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THE EXPERIMENTAL INFECTION OF PUPAE OF *PHILOSAMIA CYNTHIA* DRURY (LEPIDOPTERA: SATURNIIDAE) WITH *TRYPANOSOMA CRUZI* CHAGAS

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Trypanosoma cruzi Chagas is normally a parasite of reduviid bugs and certain mammals. In the present study, the flagellate was injected into body cavities of pupae of the moth *Philosamia cynthia* Drury in an attempt to culture the parasites *in vivo* for diagnostic purposes. Although the number of insects examined was very small, some results seemed definite enough and of sufficient interest to the biology of the parasite to warrant a report.

MATERIALS AND METHODS

The strain of *T. cruzi* used was acquired from the Army Medical School and had been maintained in our laboratory for about one year by passage through mice, *Triatoma* and culture. The culture medium used was blood agar with saline overlay (Offut, 1946). Cocoons of *P. cynthia* Drury were collected in New York during February, 1948. Normally, this moth overwinters in the pupal stage with the adults emerging around June.

A small opening was cut in each cocoon in order to observe the pupa. Using aseptic technique, normal-looking pupae were inoculated with a small-gauge hypodermic needle. After inoculation the opening in the cocoon was covered with transparent cellulose tape. Fluid for examination was withdrawn aseptically in the same manner.

The inoculum consisted of small amounts (from 0.01 to 0.1 cc.) of heavy cultures or of blood from infected mice showing tryanosomes in the peripheral circulation. The pupae were kept at room temperature varying from about 16° to 24° C. without any precautions being taken to control humidity.

The best preparations of insect blood or body fluid (these terms will be used interchangeably) were made by fixing fresh smears in osmic acid vapor for about 30 seconds, or by mixing blood on a slide with formalin and allowing the mixture to dry (Elkeles, 1945a). Giemsa solution was used to stain the slides.

Pupae that died or were sacrificed were fixed in Bouin's after slitting the exoskelton to allow the reagents to penetrate. To facilitate sectioning, the insects

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were embedded in paraffin twice. After the first embedding the paraffin was cut away and the exoskeleton was picked off with forceps and scalpel. The insect was then reembedded. Serial sections were cut 7 and 10 μ thick. Stains used for sections were: an iron alum-hematoxylin mixture (Goldman, 1949) counterstained with Van Gieson's picrofuchsin; a modified Giemsa technique (Conn and Darrow, 1946, p. $1A_2$–12); and a picric-hematoxylin method developed by the author (unpublished).

Mice used in the experiment were inoculated intraperitoneally, and tail blood was examined for trypanosomes.

EXPERIMENTAL RESULTS

Of five pupae inoculated with *T. cruzi* three became infected.

Pupa No. 1

Parasites in body fluid—This insect was inoculated on March 1, 1948 with cultured parasites. From the seventh to fifteenth day following inoculation, culture stages of *T. cruzi* (mainly crithidia of various shapes and sizes) were seen in wet mounts of insect blood. Between then and April 5 when the pupa was reinoculated with a heavy culture no organisms were found upon repeated examination. Examinations of the body fluid on the first, fourth and seventh day after reinoculation were negative for parasites. On the eleventh day many trypanosome, crithidia and leishmania forms were seen in wet mounts and stained slides.

The most striking feature seen in wet mounts was phagocytosis of parasites by hemocytes corresponding most closely to "spherule" cells (Snodgrass, 1935, p. 393). This was a consistent phenomenon observed practically every time that parasites were seen. Parasites were caught and ingested from either the flagellate or aflagellate ends, the free end continuing to writhe during this process in a typically serpentine manner (plate 1, fig. 1). As many as six or eight parasites could sometimes be seen projecting from a single cell. This process was verified as phagocytosis by stained blood smears which showed parasites in various stages of deterioration within insect cells.

Between the eleventh day, when parasites were observed, and the 27th day, when the insect was sacrificed, repeated examinations were made of the body fluid. Although it appeared that certain forms were more numerous at one time than at another, possibly indicating cyclical development, examinations were not quantitative enough to verify this impression. During this period all the classical forms of *T. cruzi* were seen: broad and slender trypanosomes, highly variable crithidia forms, and round bodies of different sizes possessing flagella of varying delicacy and length.

From time to time, blood from this pupa and others that were infected revealed trypanosomes in division (plate II, figs. 9, 10, 11). In addition one trypanosome was seen that was perfectly formed except for lack of a nucleus (plate II, fig. 12).

On one occasion two hemocytes were seen containing two and five active trypanosomes respectively. The cells were larger than spherule cells, had smooth outlines with no processes, and lacked pigment or globules. In each case one parasite was doubled back on itself so that it resembled two organisms close together. In one cell, another parasite was coiled in a rather tight knot whose loops slid

across each other sluggishly somewhat like a coiled nematode. The other trypanosomes were stretched out in the normal position. When pressure from the cover slip ruptured one cell, the released organisms could be seen swimming about freely in the surrounding medium.

In order to confirm the identity of the observed parasites as *T. cruzi,* body fluid was cultured in test-tubes and later subinoculated into a mouse. In all cases organisms of *T. cruzi* morphology were recovered with the mouse dying ten days after appearance of flagellates in its blood.

Parasites in fixed tissues.—On May 2nd, 27 days after the pupa was inoculated the second time, the insect was sacrificed and prepared for sectioning. Blood drawn at this time showed trypanosomes and very small flagellated, leishmania-like forms. The exoskeleton on one side was soft and the wing bud on that side was misshapen. Stained sections revealed widespread invasion of fixed tissues by typical intracellular leishmaniform bodies. Most of these occurred in the fat body which, at this stage in the pupal cycle, was the most prominent tissue. A few small patches of parasites were also found in other tissues.

By studying a series of fat body cells in various stages of deterioration, it was possible to draw up a theoretical cycle of lesion formation. At first, when few parasites are present, the host cell shows no reaction (plate II, fig. 8). As the parasites multiply the cell loses its original transparency and becomes more dense. Large nuclei appear around and within the infected cell. Eventually, the cytoplasm becomes opaque with a vacuolated, homogenous, dark-staining substance replacing the original cell contents (plate I, figs. 2, 4). Many insect nuclei are present and leishmania forms are scattered throughout dense material in the cell. As the cell becomes completely deteriorated and crowded with parasites some of the latter appear to spread into adjacent healthy tissue presumably causing a repetition of the process just described.

This pathology was peculiar to fat body. In other tissues, there was no great proliferation of parasites and no obvious production of lesions (plate I, fig. 3). Flagellated forms were not observed in any lesions that occurred either in fat body or other tissues.

Parasites in midgut.—In this species of moth the midgut exists for a while in the pupa as a sac full of plant debris. Sections of this portion of the pupa revealed innumerable flagellates, mainly crithidia stages (plate I, fig. 5). A few appeared to be in the leishmania-crithidia cycle and, rarely, trypanosomes were seen. Parasites were either embedded in gut contents or massed in spaces between plaques of debris, giving an impression of rapid and repeated multiplication. Many appeared to be attached to the epithelium and in some areas a distinct invasion of gut wall had occurred (plate I, fig. 6, 7).

Pupa No. 2

Parastes in body fluid.—This pupa was inoculated on March 1 with cultured *T. cruzi.* Culture-type flagellates were first seen in the blood eight days after inoculation and persisted until about fifty days later. As in pupa No. 1, there appeared to be a cyclical prevalence of different stages. Phagocytosis was common and followed the pattern described for pupa No. 1. Cultures of body fluid showed a heavy growth of organisms.

On April 28, almost two months after inoculation, body fluid was negative for parasites both by direct examination and by culture. Instead of being clear and serum-colored as before, the blood was milky and full of globules and amorphous particles. On May 17, when the pupa was opened, fat body and midgut were both less prominent than those in pupae dissected earlier in the year. Smears prepared from body fluid showed no bacteria, few hemocytes and many scales like those found on adult moths. No parasites were seen.

Parasites in fixed tissues.—Twelve days after inoculation a bit of tissue was aspirated from the insect while blood was being drawn for direct examination. Some of the cells contained active flagellated parasites. In others there occurred rounded aflagellated bodies about 7 μ in diameter containing refractile granules. Occasionally the entire body would rotate as a unit and, from time to time, the periphery of these bodies would show rapid, shallow undulations.

Stained sections prepared after the death of the pupa on May 17 were negative for parasites.

Pupa No. 3

Parasites in body fluid.—This insect was inoculated on March 1 with blood from an infected mouse. Smears made eight and twelve days later were negative for parasites. On the seventeenth day many crithidia and a few leishmania and trypanosome forms were seen. Dividing forms and phagocytosis were very evident.

In addition to readily recognizable stages, stained slides revealed forms of unknown classification (plate II, figs. 13–20). These were oval bodies 5–8 μ by 2.5–3.5 μ. In only one case (plate II, fig. 13) was a flagellum-like structure present. The kinetoplast generally appeared as a bright red rod next to which was a more lightly stained nucleus. The blue cytoplasm contained few to many globules of varying sizes of the same color as the kinetoplast. One similar body was seen on a slide prepared from pupa No. 1 (plate II, fig. 21).

This pupa was not fixed for sectioning.

Negative and Control Pupae

Two pupae were inoculated with infected mouse blood on March 1 and March 23 respectively. One was reinoculated with blood on April 27. Smears and tissue sections remained negative for parasites in both insects. Of four controls used, two were left in New York to develop without any interference. The other two were brought to Atlanta with the rest of the experimental pupae. Of these, one was examined at intervals for naturally occurring flagellates. No flagellate parasites were seen in the insect that was examined and all pupae developed into adults.

DISCUSSION

Faria and Cruz (1927) reported intracellular leishmania and trypanosome forms of *T. cruzi* in epithelial cells of the gut of infected *Triatoma.* This observation has not been confirmed by later workers and, at present, the life cycle of *T. cruzi* in *Triatoma* is not believed to include an intracellular phase.

Dias (1934) and Hoare (1938) inoculated *T. cruzi* into body cavities of the bug, *Triatoma,* and the wax-moth caterpillar, *Galleria mellonella,* respectively. In both cases a culture-tube type of development was reported. Dias studied stained

sections of various organs, but did not observe intracellular leishmania. Hoare's paper gives no details of technique, but again there is no mention of intracellular leishmania. Wood (1942), examining dead or laboratory killed *Triatoma,* found many *T. cruzi* flagellates in the body fluid of some of his insects. However, no invasion of fixed tissues by the parasite seems to have occurred.

As far as can be determined, the present paper is the first report of the development of *T. cruzi* into a leishmaniform, tissue-invading stage in an unnatural, invertebrate host. This information may be of some significance in considering relationships in this group of flagellates. In this connection, growth of the parasite in the gut of the pupa is also of interest. Although *T. cruzi* ordinarily develops in positions that are bathed in blood or other animal fluids, it has here been seen to proliferate enormously in gut contents of an exclusively plant-feeding insect.

The absence of dividing trypanosomes in the peripheral blood of infected vertebrates is considered a valuable diagnostic feature for *T. cruzi* (Craig, 1948, p. 203). That dividing trypanosomes may occur in this species, however, has been indicated most recently by Meyer (1944), Elkeles (1945b), Chang and Negherbon (1947) and Meyer and Xavier de Oliveira (1948). In a study of mammalian trypanosomes, Hoare (1936), was able to fit *T. cruzi* methods of reproduction into a pattern formed by other species he was considering. Observations made in the present study tend to confirm Hoare's thesis, namely: that *T. cruzi* reproduces primarily in the leishmania and crithidia stages, but may also reproduce occasionally in the trypanosome stage.

The technique reported here for study of *T. cruzi* would appear to be a fruitful approach to the study of other parasites. Insects are small, easily handled and have a relatively simple internal organization. The entire blood supply may be examined and the complete animal sectioned and stained. Under such conditions host-parasite relationships may be more easily investigated than when more usual laboratory animals are used.

SUMMARY

1. *In vivo* culture of *T. cruzi* within pupae of a saturniid moth is reported.

2. In addition to development of usual extracellular insect stages, the parasite showed intracellular stages similar to those found ordinarily only in vertebrate hosts.

3. Large numbers of parasites also developed in the mid-gut of this exclusively plant-feeding insect.

ACKNOWLEDGMENT

It is a pleasure to thank the following members of the Communicable Disease Center staff for their cooperation as indicated: Dr. M. M. Brooke for general direction and criticism; Dr. Harry Pratt for assistance with the entomological aspects; Miss Lois Norman for providing cultures and culture media; and Miss Sadie Johnson for technical assistance. Determination of moth pupae and adult moths was made by Mr. H. W. Capps of the U. S. Department of Agriculture.

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Explanation of Figures

Plate I

Fig. 1. Trypanosome in body fluid, partially phagocytized by hemacyte. Wet mount, ×2250.

Fig. 2. Lobes of fat body, showing one heavily parasitized cell; smaller, dark-staining bodies are nuclei of fat body cells. Picric-hematoxylin, ×320.

Fig. 3. Parasitized tissue other than fat body. Two leishmania forms are shown adjacent to an insect nucleus. Surrounding tissue appears normal. Hematoxylin-Van Gieson, ×2000.

Fig. 4. Parasitized cell from fig. 2 under higher magnification. Picric-hematoxylin, ×1250.

Fig. 5. Numerous flagellated stages in midgut. Kinetoplasts are stained darkly and are more or less central in torpedo-shaped organisms. Nuclei are lightly stained and do not show well. Giemsa, ×1805.

Fig. 6. Section of midgut epithelium. Lumen is at right. Epithelium detached from basement membrane during sectioning. Area marked off indicates point of greatest penetration by parasites. Giemsa, ×215.

Fig. 7. Marked off area from fig. 6 enlarged to show crithidiaform organisms penetrating epithelium. Lumen is at upper right. Flagella of all organisms in tissue point away from lumen. Along lumen surface are seen flagellates and leishmania (lower right, dividing form). Giemsa, ×1080.

Plate II (All magnifications somewhat greater than indicated due to enlargement in engraving.)

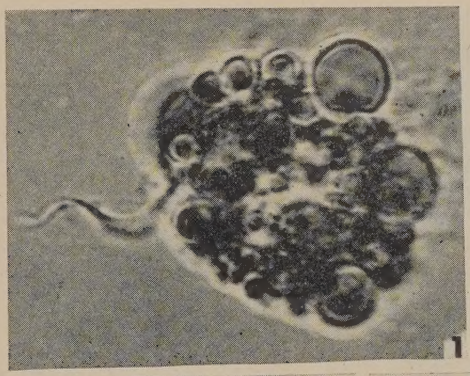
Fig. 8. Early stage in infection of fat body cell; no pathological changes are apparent yet. One leishmania form shown. Hematoxylin-Van Gieson, ×1350.

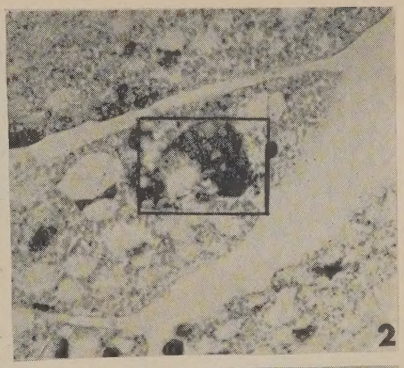
Fig. 9–11. Dividing trypanosomes. Giemsa, ×2615.

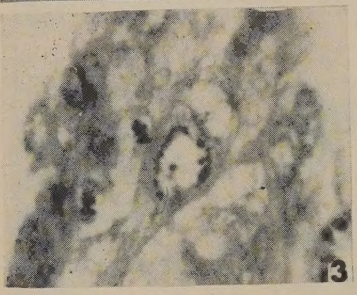
Fig. 12. Trypanosome with no nucleus. Giemsa, ×2745.

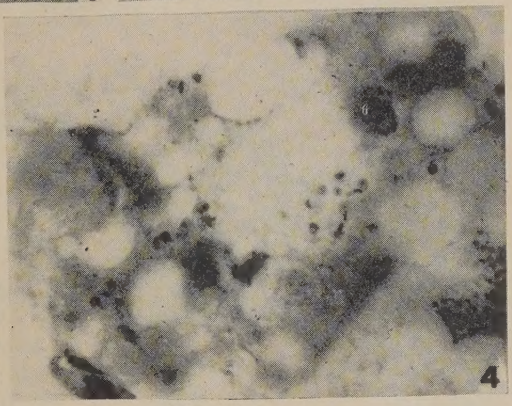
Fig. 13–21. Forms of unknown classification. Giemsa, ×2350. In fig. 13 arrow points to tenuous, flagellum-like projection.

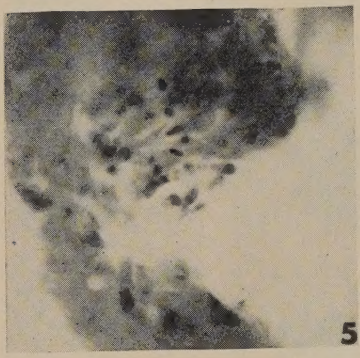
PLATE I











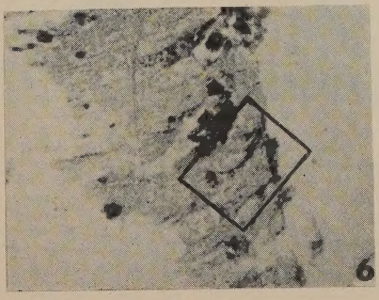
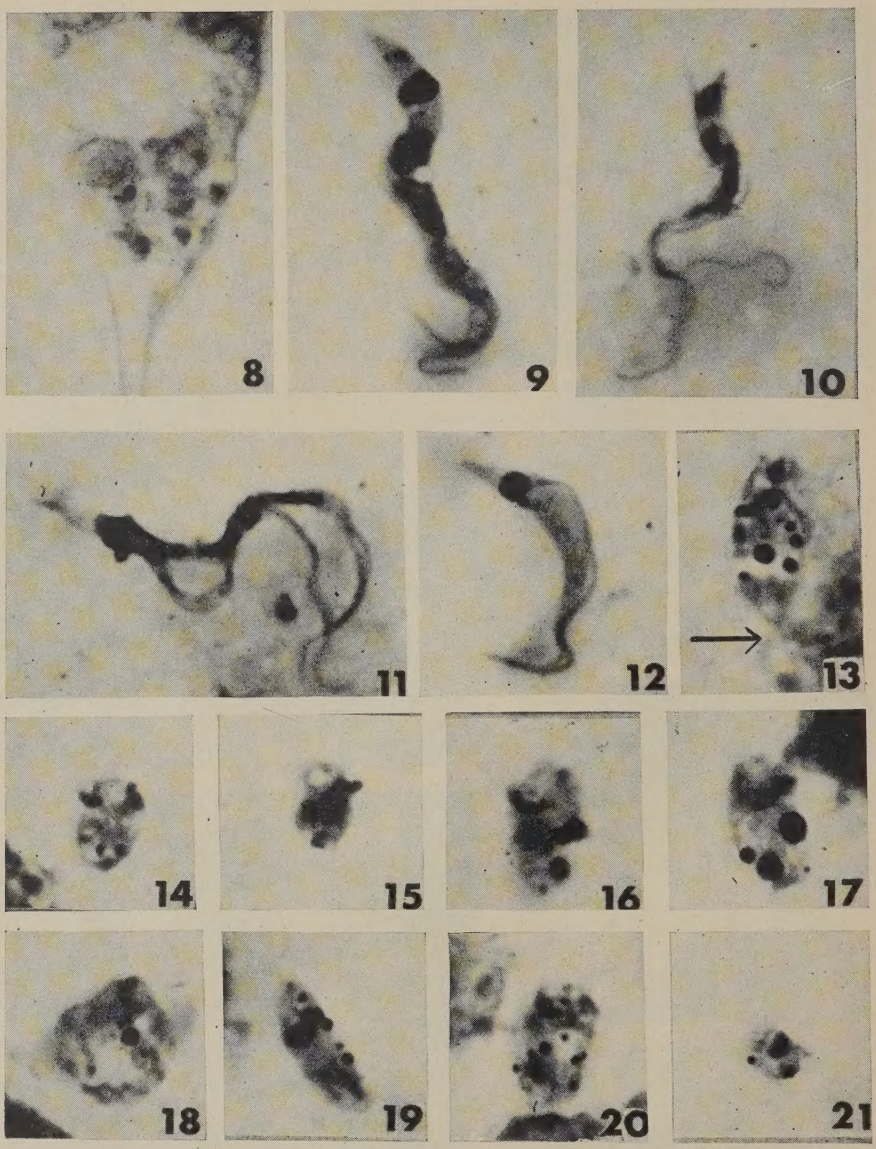


PLATE II



CESTODES OF CALIFORNIA GULLS

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There is but little information available regarding the helminthology of the California coast; therefore the following account of some cestodes found in gulls of this region may be of interest.

Sixty-six birds were examined, fifty-six of which contained one or more parasites, an infection of 84.8%.

The worms have been examined in both fresh and preserved material, in whole mounts and sections. The fixatives employed were alcoholic-sublimate-acetic and alcoholic Bouin, with a few specimens fixed in 10% formalin. The stains used were aceto-carmine and Ehrlich's hematoxylin for whole mounts, and for sections the latter and iron alum hematoxylin.

All measurements in this paper are expressed in mm. and were made on preserved material except as stated in the text.

DESCRIPTION OF SPECIES

Tetrabothrius lari. This worm is by far the commonest species in the western gull (*Larus occidentalis*) though occurring occasionally in the California and ring-billed gulls (*L. californicus* and *L. delawarensis*). It was found also in two of three specimens of glaucous-winged gulls (*L. glaucescens*) and in a single specimen of the herring gull (*L. argentatus*).[1] It occurs throughout the gut, but mainly in the upper half thereof. Of twenty-five western gulls examined, only four were free from this parasite. It was found in seven of twenty-four California gulls and in four of eleven ring-bills.

There are some minor differences between my specimens and those of Yamaguti, who described this species in 1935. He stated (l.c., p. 184) that the inner longitudinal muscle bundles have "as many as 70 fibres each." In my material the maximum number counted was sixty-two, with a range from eleven to sixty-two. This variation agrees with that found by Linton (1927) in this genus.

The eggs in my specimens average somewhat smaller (.055 × .031) as compared with .048–.072 × .036–.048 in those of Yamaguti, which are "subglobular" while mine are ovoid. Also the diameter of the cirrus sacs in my specimens (.049) is somewhat smaller than in *T. lari* (.06–.08). These differences however are insufficient to warrant separation of the two species.

So far as the writer knows this species has hitherto been recorded only in *L. canus major*. The wide range of this bird on the coasts of Asia from Kamchatka to Formosa and of other species of gulls in both North America and Asia however readily explains its presence in gulls on both shores of the Pacific.

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[1] Only one or two specimens in one bird which was probably a herring gull. This bird was immature and the difficulty of identifying gulls in immature plumage renders its identification somewhat uncertain.

Paricterotaenia Fuhrmann, 1932

There are two species of this genus in the gulls of the region, one of which is apparently *P. porosa* and the other *P. ransomi,* although differing from the published accounts of these species in certain minor details.

Paricterotaenia porosa (Rudolphi, 1810) Fuhrmann, 1932

I have three records of this species, one from a California gull and two from ring-billed gulls; and two probable records, one from a ring-billed and one from a Bonaparte's gull. My specimens agree in the main with the descriptions of Cohn (1901) and Krabbe (1869), though the diameter of the cirrus sac is less in my specimens than in Cohn's material. At its poral end this organ shows a distinct enlargement (.045–.050) in diameter. It then narrows but forms another enlargement (.040) at its termination near the middle of the segment, where it is more or less completely surrounded by the coils of the vas deferens as described by Cohn.

The slightly lobed vitellarium is somewhat obliquely situated as described by Cohn, but his "tiefe Taschen nach vorn und hinten" (l.c., p. 371) are not present. Both Cohn and Linton (1892) described the cirrus as short and smooth. They gave no measurements, so that their statement of size is not definite. In my worms the cirrus varies in length from less than .070 to .150 depending of course on the degree of extension. It is smooth and directed forward as Linton observed. The number of testes is somewhat less in my specimens (20–30) than that given by Cohn (40–50).

The differences described above from the descriptions of Cohn, Krabbe and Linton do not seem to warrant the separation of my species from *P. porosa.*

Paricterotaenia ransomi (Linton, 1927) Fuhrmann, 1932

Of more frequent occurrence in the gulls examined is another species of *Paricterotaenia* which closely resembles *P. ransomi,* described as *Choanotaenia ransomi* by Linton (1927) from the loon (*Gavia immer*) and from several species of gulls. Other than very minor differences in measurement, the two species appear to be identical. It was fairly common in the ring-billed gull, occurring in three of eleven birds examined, while it was present in four of twenty-five western gulls, and what is probably this species was found in one specimen of Heermann's gull (*L. heermanni*), but the poor condition of the only scolex I possess renders this determination somewhat uncertain.

This genus is represented by what is apparently a single species,[2] which occurred

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Hymenolepis californicus

Description of figures

All figures were outlined by camera, with details added free hand.

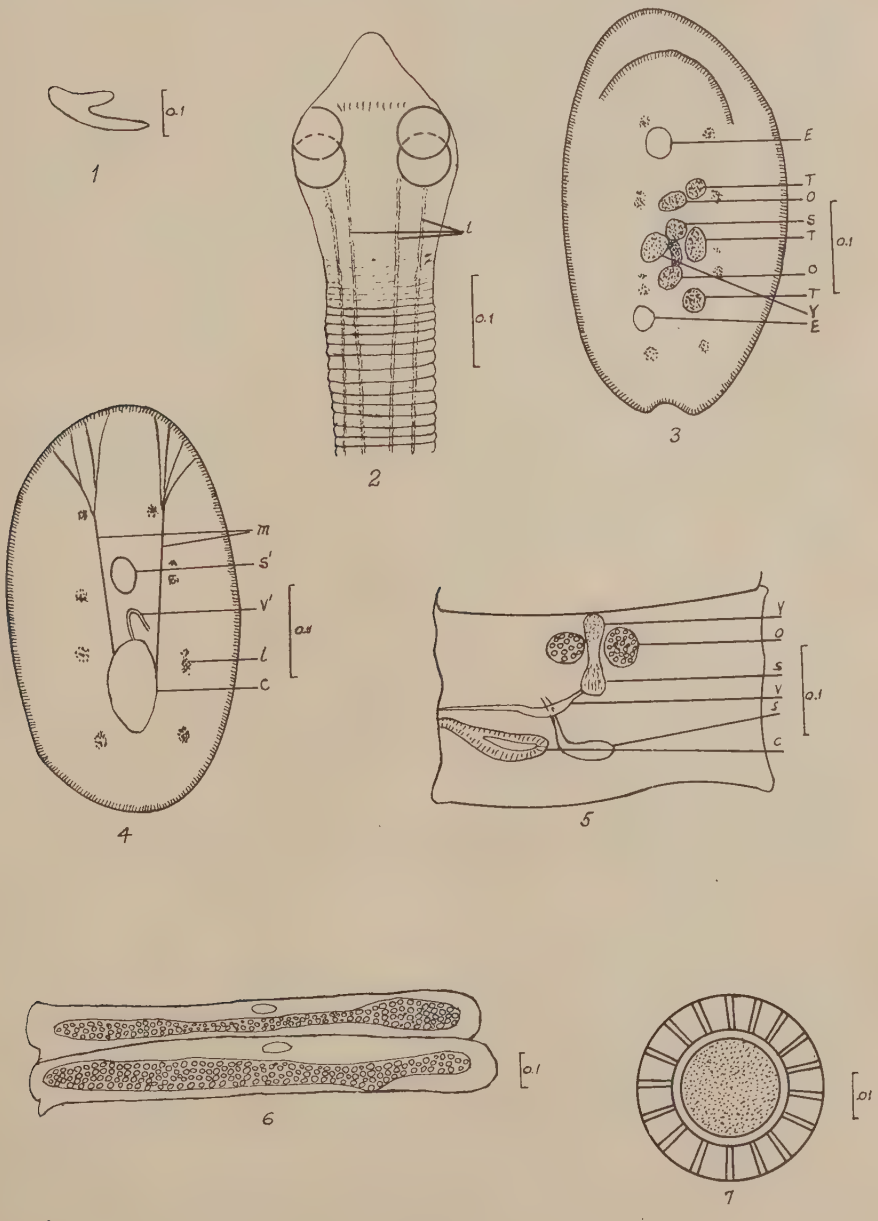
FIG. 1. Rostellar hook. FIG. 2. Scolex and neck, partly contracted. FIG. 3. Cross section of proglottid, showing sex glands. FIG. 4. The same, showing cirrus pouch and muscles. FIG. 5. Whole mount of proglottid, showing sex organs. FIG. 6. The same, showing uterus. FIG. 7. Cross section of cirrus pouch showing muscles.

C, Cirrus pouch; E, Ventral excretory vessel; 1, Inner longitudinal muscle; m, Muscle cords of cirrus pouch; O, Ovary; S, Seminal receptacle; S′, External seminal vesicle; T, Testes; V, Vagina; V′, Vas deferens; Y, Vitellarium.

[2] What may be another species occurred in one of the ring-bills, but condition of the specimens and lack of scolices renders this uncertain.

Hymenolepis

in thirteen of twenty-four specimens of the California gull and four of eleven ring-bills. It differs from any species hitherto described in its combination of characters and it is accordingly described as new.



FIGS. 1–7

Hymenolepis californicus n. sp.

(Figs. 1–7)

With the characters of the genus.

Length 45–150 mm. in extended fresh specimens, exceptionally up to 240 mm. Width 1–1.5 in the same. Proglottids wider than long; numerous, about 1200 in a 70 mm. specimen. Neck 0.2–1.0 long by .05–.128 thick. Scolex .100–.208 in diameter. Suckers average .075. Adult proglottids .11–.26 long by 1.04–1.53 in fresh specimens. Hooks 10, in a single crown, which average .016 in length, with a hilt about twice as long as the handle and one-half as long as the blade. Longitudinal muscles in two layers, the inner consisting of four heavy bands (dorsal and ventral), with 2–3 bundles in each band, the fibres of which frequently cross from one bundle to another, and with an occasional anastomosis between the bands. The outer layer comprises 30–40 small bundles of 1–2 fibres each, which do not cross over. Genital atrium wide and shallow, receiving the vas deferens and vagina, the latter opening posterior and ventral to the former. The former is surrounded by a thick muscular sac which is attached to the aporal side of the proglottid by 2 strong muscle cords, and which averages .156 × .032. The extent of this sac in the proglottid depends on the state of expansion or contraction of the latter. Internal and external seminal vesicles connected by a narrow part of the vas deferens, which follows a straight or sinuous course depending on the form of the proglottid. The external vesicle averages .085 × .031. Testes 3, equally spaced, located dorsal to the female genitalia and averaging .04 × .03. Seminal receptacle .047 × 0.29. Ovary bi-lobed, the lobes averaging .045 × .056 × .035 and sub-divided into a few minor lobes. Vitellarium, dorsal to the ovary and between its lobes, averages .036 × .028 × .02. Uterus sac-like, filling the proglottid. The eggs in fresh specimens average .13 × .08. The embryo averages .05 × .03, the embryonic hooks .008.

An interesting feature of this study is the considerable specificity in the relation of parasite and host. *Tetrabothrius lari* was found mostly in the western and glaucous-winged gull,[3] while *Hymenolepis* occurred only in the California and ring-billed gull. The number of specimens of *Paricterotaenia* is rather small to admit of any definite conclusions regarding their distribution but apparently they occur indifferently in all.

SUMMARY

The foregoing paper gives an account of the cestodes found in seven species of gulls on the California coast. It extends the range of *Tetrabothrius lari* from the western to the eastern shores of the Pacific ocean, and of *Paricterotaenia ransomi* from the Atlantic to the Pacific coast of North America.

A new species, *Hymenolepis californicus* is described, and the distribution of parasites with respect to host is discussed.

I wish to thank the San Diego Zoological Society for the use of a room during this study, and especially Dr. Arthur L. Kelly and other members of the staff for many courtesies rendered.

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[3] Of the latter species only three specimens were examined, two of which harbored this parasite.

A SURVEY OF INTESTINAL PARASITES IN DOMESTIC RABBITS IN SIX COUNTIES IN SOUTHERN CALIFORNIA[1]

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The number of intestinal parasites reported from hutch-raised domestic rabbits is not large, and of these only a few are encountered with sufficient frequency to permit any calculation of incidence of infection. However, even for those parasites of most frequent occurrence, the coccidia, there is little reliable information concerning their actual prevalence in rabbits maintained for commercial purposes.

No part of the United States embraces such a concentration of domestic rabbits as does the Los Angeles area in southern California. For this reason, and because of its proximity to the laboratories of the U. S. Rabbit Experiment Station, this area was selected for studies of the enterozoic parasites of the domestic rabbit.

Between January 28 and March 19, 1948 a survey was made to determine the prevalence of intestinal parasites, particularly coccidia, in hutch-raised domestic rabbits in southern California. Altogether 1,200 fecal specimens were collected in 23 commercial rabbitries in 15 localities scattered over parts of Los Angeles, Orange, Riverside, San Bernardino, San Diego and Ventura Counties.

MATERIALS AND METHODS

The size of the rabbitries visited in making this survey ranged from about 30 does to more than 300. In the smaller rabbitries fecal samples were collected under each occupied hutch, but in the large units random sampling was used. The number of specimens from any one rabbitry ranged from 29 to 80. Each sample consisted of half a dozen or more fecal pellets collected in a paper cup. Such a sample obviously represented the hutch, as a unit, rather than a single individual. However, the two would be the same except in the case of does with litters out of the nest box, or in the case of orphan litters; and even in such instances there are distinct advantages in considering the occupants of one hutch as a unit. Animals occupying the same hutch show similar infections more commonly than is the case with segregated animals. It would, therefore, be unfair to consider each of the several animals sharing one hutch as a distinct statistical entity. Furthermore, it would be manifestly impractical to attempt to obtain samples representative of individual members occupying a hutch in common.

Actual oocyst counts were not attempted, but to insure reasonable uniformity in estimating oocyst frequency, approximately 50 cc. of liquid was added to an estimated 2 gms. of fecal material. Tap water was used in preparing suspensions for immediate examination, but 2% potassium bichromate solution was used if samples were to be held overnight. A drop of the suspension of macerated fecal material sufficient to form a film under a 22 mm. square cover glass was then studied, and

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[1] From the U. S. Rabbit Experiment Station, Bureau of Animal Industry, Agricultural Research Administration, U. S. Department of Agriculture.

each of the five species of coccidia was recorded separately. The following arbitrarily assigned system for evaluation of oocyst frequency was employed:

Trace—1 to 3 oocysts of any one species in 10 fields with the 16 mm. objective; light—up to an average of 1 oocyst per field; moderate—up to an average of 3 per field; numerous—up to about 10 per field; heavy—more than 10 per field.

Such an evaluation is purely relative, and would not be applicable to situations in which a greater range of intensity of infection is encountered, as in the case of experimentally induced infections or severe ones of natural origin.

TABLE 1.—*Incidence of the various species of intestinal parasites, presented for individual rabbitries and for the entire survey**

Rabbitry	Number of samples	Number of samples positive for coccidia—by species					Total positives, any coccidia		Frequency of single and multiple species infections Species			*Passalurus ambiguus* (pinworms)	
		E. magna	*E. media*	*E. perforans*	*E. irresidua*	*E. stiedae*	Number	Approximate %	1	2	3	Number	Approximate %
I	73	4	7	1	2	..	13	18	12	1	..	7	10
II	37	..	1	..	4	1	5	14	4	1	..	..	..
III	32	6	1	1	2	..	8	25	6	2	..	..	..
IV	45	4	4	1	..	..	8	18	7	1	..	..	..
V	68	9	16	4	10	5	31	44	19	11	1	18	26
VI	60	7	5	3	2	1	14	23	11	2	1	5	8
VII	61	..	..	4	..	..	4	7	4	..	..	5	8
VIII	64	1	1	1	2	1	4	6	3	..	1	..	..
IX	56	1	..	..	..	..	1	2	1	..	..	..	..
X	43	1	2	..	1	..	4	9	4	..	..	..	..
XI	44	2	3	2	1	..	7	16	6	1	..	..	..
XII	43	1	2	..	4	..	6	14	5	1	..	1	2
XIII	54	6	3	1	1	..	10	19	9	1	..	3	6
XIV	55	10	4	1	2	..	15	27	14	..	1	5	9
XV	29	5	1	..	1	..	6	21	5	1	..	1	3
XVI	44	9	2	..	..	..	10	23	9	1	..	..	..
XVII	40	2	6	..	..	1	8	20	7	1	..	..	..
XVIII	80	7	6	1	9	..	21	26	19	2	..	1	1
XIX	66	..	..	..	2	..	2	4	2	..	..	20	37
XX	59	..	..	..	1	..	1	2	1	..	..	..	..
XXI	47	5	..	..	..	..	5	11	5	..	..	2	4
XXII	48	10	6	7	2	2	17	35	10	4	3	4	8
XXIII	52	1	2	..	2	..	5	10	5	..	..	..	..
Total No.	1200	91	72	27	48	11	205	..	168	30	7	72	..
Average %	....	7.6	6.0	2.3	4.0	0.9	...	17	...	..	..	..	6

* One specimen from Rabbitry XIV contained eggs identical to those of *Obeliscoides cuniculi*, the American stomach worm of rabbits, and presumed to be so.

RESULTS

Table 1 shows the distribution of parasites, as detected by direct examination of a single fecal specimen from each hutch. The total incidence of coccidia, all forms considered, varied in different rabbitries about 2% to 44% with an overall average of 17%. In this particular survey *Eimeria magna* was first in frequency, with an incidence of 7.6%, while *E. stiedae* showed the low frequency of 0.9%. This latter species is well recognized by the breeders, causes economic losses because livers of affected animals cannot be marketed, and the disorder is easily controlled by good management. All of these circumstances doubtless operate to keep the incidence of this species low.

Pinworms, *Passalurus ambiguus,* were detected in droppings from 6% of the hutches, and were found in 12 out of 23 rabbitries. Since fecal examinations may

be unreliable in the detection of pinworms, the actual incidence could have been considerably higher than that recorded, and more than 12 of the rabbitries may have harbored the parasite. Curiously enough, the rabbitry showing the highest incidence of *Passalurus* (Rabbitry XIX with 37%) was one of the lowest in incidence of coccidiosis, whereas the only other rabbitry (No. V) with a very high incidence of pinworms had coccidia with the greatest frequency of any in this study. Neither of these rabbitries had experienced excessive mortality in the last few weeks before the survey, but reliable figures on growth and weaning weights were not available.

One specimen in the 1,200 contained a few eggs of what was probably *Obeliscoides cuniculi,* the American stomach worm of rabbits.

Efforts to associate the incidence of infection with the geographical location, temperature, altitude and other physical factors led to no positive results, probably because of two principal circumstances. With the exception of Rabbitry XIII, discussed individually below, the climatic conditions of late winter and early spring were quite similar throughout most of the area surveyed, thus tending to promote uniformity. Then, too, differences in management practices doubtless tended to overshadow variations resulting from other causes.

Rabbitry No. XIII was in the mountains at an elevation of about 4,600 feet. Midday temperatures permitted some thawing in areas exposed to direct sunlight, but throughout much of the day temperatures were below freezing, and snow blanketed the ground. In the rabbitry, which was protected from snow, all but the freshly fallen droppings were frozen, and sporulation of oocysts would have been very slow at best. In this rabbitry the incidence of intestinal coccidia was 19% with *E. magna* predominating, and *E. media* second in prevalence. Both mortality and morbidity with mucoid enteritis (clinical diagnosis) were average or more.

Of the 205 specimens positive for coccidia, 168 showed but one species; 30, two; and 7, three species. Table 2 shows the observed number of two-species infections with each combination of coccidia, and gives the ratio of the observed frequency of each association to the theoretical frequency of the same association calculated on the basis of incidences found in this study. Actually, three contributing factors account for the discrepancy between the theoretical frequency of association and that observed. They are as follows:

1. The samples are small, and close approximation of mathematical probability is not to be expected.

2. Double infections were somewhat more numerous, proportionately, in rabbitries with higher individual incidences than in those with lower values, and the process of averaging tends to obscure these deviations.

3. Conditions favorable to the dissemination of one coccidium are likewise favorable to the spread of the others, so a "better-than-chance" association is to be expected.

Triple infections are uniformly recorded as the three double infections into which each may be resolved. It is because of this circumstance that the total number of two-species associations represented in table 2 exceeds the total number of double infections of table 1 by three times the number of triple infections also reported in table 1.

Table 3 shows the relationships between the number of species of coccidia present in the rabbitry, the overall incidence of coccidiosis, and the percentage of double

TABLE 2.—*Association of parasites in two-species infections*

Organisms	Observed number in 1,200 samples	Ratio of observed to theoretical association
Eimeria magna and *E. media*	15	2.7
E. magna and *E. perforans*	7	3.4
E. magna and *E. irresidua*	4	1.1
E. magna and *E. stiedae*	..	..
E. media and *E. perforans*	6	3.6
E. media and *E. irresidua*	7	2.4
E. media and *E. stiedae*	3	4.6
E. perforans and *E. irresidua*	4	3.7
E. perforans and *E. stiedae*	2	7.9
E. irresidua and *E. stiedae*	3	6.9
All associations with two species of coccidia	51	2.7
Coccidia (any species) and pinworms	17	1.4

and triple infections. Actually, the prevalence of multiple infections is a function of both the number of species and the incidence of these species individually rather than collectively, so table 3 is only indicative of tendencies. Nevertheless, it is apparent that measures which tend to keep the incidence of coccidiosis low also operate to keep the number of species represented small, all of which circumstances tend to decrease the prevalence of multiple infections.

From Table 4 it will be noted that the relative abundance of oocysts found in fecal specimens tended to assume a similar pattern for all species. Throughout, extremely light concentrations of oocysts prevailed, and heavy concentrations such as appear in animals experimentally infected with very modest numbers of oocysts were entirely absent. *Eimeria stiedae,* with a very low incidence of infection, was also found to show the greatest proportion of extremely light concentrations of

TABLE 3.—*Incidence of multiple species infections in relation to total herd incidence and total number of species present*

Number of species of coccidia present in rabbitry	Number of rabbitries in group	Average incidence of coccidiosis for all rabbitries in group—%		
		All infections	Two species	Three species
1	5	4.5	..	..
2	1	22.7	2.3	..
3	7	14.5	1.7	..
4	6	21.9	2.1	0.3
5	4	27.5	7.1	2.5

oocysts. The stern measures of control exercised by breeders have been largely responsible for both of these circumstances. Variations in patterns found for other species are only in part attributable to the small numbers of samples, and are related also to differences in life histories, interacting with climatic factors, management practices and a number of circumstances too involved for presentation here.

TABLE 4.—*Relative abundance in fecal specimens of oocysts of each species of coccidium*

Organism	Incidence (% of 1,200 cases)	% of total positives in each category			
		Traces	Light	Moderate	Numerous
E. magna	7.6	48.3	29.7	19.8	2.2
E. media	6.0	65.3	23.6	9.7	1.4
E. perforans	2.3	59.3	25.9	11.1	3.7
E. irresidua	4.0	58.3	22.9	18.8	..
E. stiedae	0.9	72.7	18.2	9.1	..

Within the range of infection represented in this study, any relationship that exists between incidence of infection and oocyst concentration is largely lost through the process of averaging. Inasmuch as the analysis of such a relationship requires the grouping of data in another manner, this aspect is not being presented in this paper.

DISCUSSION

The average incidence of 17% for coccidia of all species combined was substantially lower than has been recorded or reported hitherto for observations made at a corresponding season. In the winter of 1944–45 the herd at the U. S. Rabbit Experiment Station, Fontana, California, showed an average total incidence of approximately 40%, which was essentially the same as the average of the incidences found in scattered commercial rabbitries in this general area. In December 1945 a single commercial rabbitry in Fontana was found to have a total incidence of 60%, with *E. magna, E. media* and *E. perforans* present in 35, 25, and 30% of the cases, respectively. In this same study 37% of all positives were double infections, and 7% were triple infections.

From these considerations one may conclude that the incidence of coccidiosis in the winter of 1948 was sufficiently lower than that of previous years when studies were made to justify some explanation. There has doubtless been some improvement in management practices; and this has played a part in keeping the incidence of infection low. In some cases, however, neither the equipment nor the method in use in 1948 differed appreciably from that employed 4 years ago, but coccidiosis was less prevalent than before. During much of the winter of 1948 California suffered one of its most severe droughts in recent years. When this survey was begun, the precipitation at Fontana for the entire season had been but 2.27 inches, and only 2.38 inches fell between the opening of the survey and March 14, five days before its close. Any rainfall within the last 5 days of the survey could not have influenced the incidence of coccidiosis because this period is not equal to the sporulation time plus the prepatent period for any coccidium encountered. Throughout the winter much of the area covered by this survey was subjected frequently to strong, drying winds. Both the lack of precipitation and the drying action of such winds are unfavorable to the survival of oocysts through sporulation. The rainfall at Fontana during the 1944–45 season was 16.98 inches, and in December 1945, between 4½ and 5 inches of precipitation had been recorded for the first 2 months of the wet season. It is probable that the low average incidence of coccidiosis prevailing during the period of the 1948 survey was largely occasioned by climatic conditions unfavorable to the transmission of the parasite.

It should be clear from the present study that there is no normal or average incidence for any species of coccidium, except as an average value for any particular sampling is determined. Kessel and Jankiewicz (1931) in a study of "more than two thousand rabbits" over a period extending "from June 1928 to September 1929" reported incidences as follows: *Eimeria magna* 19%; *E. media* 12%; *E. perforans* 30%; *E. irresidua* 10%; and *E. stiedae* 9%. One cannot determine definitely whether or not each incidence is for the entire group of animals, because many of the specimens were employed particularly for studies of liver coccidiosis, and were subjected to procedures peculiar to those studies. Actually, that is of no real consequence. The authors recognized that the incidence cited in each case was to be con-

strued only as that of their particular sample. Statements such as "in this study" and "in our survey" appear in each instance. However, their figures have been widely quoted, sometimes without the qualifying phrases of the authors. Becker (1934) unfortunately stated "The incidence in California is 12 per cent" (*E. media*), etc., which by implication might lead one to an erroneous conclusion.

In several of the rabbitries included in this survey accurate mortality records were not kept, but in most cases the hutch cards indicated the number of young born or retained, as well as the number weaned. Moreover, the trained observer can judge reasonably well by the number and appearance of the unweaned young the approximate extent of the most common disorder, mucoid enteritis. With these indications to confirm the reports of the growers, an effort was made to determine the prevalence of mucoid enteritis or other enteric disturbances in the few weeks prior to the time at which droppings were collected. Rabbitry XX, which showed the lowest incidence of coccidiosis, had experienced a very low mortality. However, it is a herd of Angora rabbits, which, characteristically, consists largely of woolers, and litters are few. Under such circumstances total mortality is usually low. Rabbitry IX, which produces animals for meat and fur, has been studied on several occasions during the past season, and found on all examinations to have a very low incidence of coccidiosis. Nevertheless, cases of mucoid enteritis were in evidence at each visit, and only 2 weeks before the sampling used on this survey was taken, several animals had died with enteritis. Four very severe cases were brought to our laboratories for examination, and all were negative for coccidia.

Throughout this study it was impossible to find a relationship between the observed or reported relative morbidity or mortality and the incidence of coccidiosis. This would tend to indicate that, at least at low levels of infection, coccidiosis cases are subclinical and unrelated to mucoid enteritis. Both of these circumstances are in agreement with the findings for routine observations on the Station herd. Such observations indicate that the incidence of intestinal coccidiosis fluctuates with the seasons, and without apparent reference to the much smaller and irregular fluctuations in enteritis mortality.

SUMMARY AND CONCLUSIONS

1. The herd incidence of any or all species of coccidia was subject to much variation.

2. There was a tendency for infections with the various species to be associated more frequently than chance distribution alone would require.

3. All cases observed in this study were subclinical.

4. The vast majority of fecal specimens yielded light oocyst concentrations or mere traces. None were heavy.

5. Infections with *Eimeria stiedae,* the cause of coccidiosis of the liver, were very infrequent.

6. There was no constant relationship between the incidence of coccidial infection and the morbidity or mortality reported for the rabbitries. The greatest single cause of mortality, as reported or observed, was mucoid enteritis, and this survey failed to demonstrate a direct relationship between that discorder and intestinal coccidiosis or other parasitism as encountered in this study.

7. The incidence of coccidiosis was lower in this part of California in the winter of 1948 than at a similar season in previous years in which studies were made. This lower incidence is attributed to the scanty rainfall and the prevalence of drying winds, both of which factors are unfavorable to the sporulation and preservation of oocysts.

8. The only intestinal parasites other than coccidia that were detected in fecal specimens examined in this study were *Passalurus ambiguus,* the pinworm and, in a single instance, the eggs of what was probably *Obeliscoides cuniculi,* the American stomach worm of rabbits.

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TWO NEW SPECIES OF HELMINTHS FROM THE CORMORANT, *PHALACROCORAX AURITUS**

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On November 17, 1947, two cormorants, *Phalacrocorax auritus,* were sent to Dr. Lyell J. Thomas for study. These birds were shot five miles northwest of Grafton, Illinois on the Illinois River and brought over by Dr. H. B. Mills, Chief of the Illinois State Natural History Survey. With the permission of Dr. Thomas the writer examined one of them for parasites and recovered, besides other helminths, four *Opisthorchis* in the liver and two pairs of *Syngamus* in the trachea which are described here as new species.

Opisthorchis vitellatus sp. nov.

(Figs. 1–4)

Specific diagnosis: Elongate, flesh-colored worms. Body, 3.45–4.03 mm long, 0.43–0.53 mm wide, with minute, conical, blunt-pointed spines, about 8 μ in length, in alternate rows, from anterior end to level of posterior testis. Circular muscles weak, longitudinal and diagonal muscles well developed. Oral sucker terminal, 0.28–0.30 mm in diameter. Acetabulum 0.20–0.24 mm, at posterior portion of anterior fourth of body. Prepharynx absent; pharynx small, oval, 57–63 μ wide, musculature weak. Oesophagus short, about 2 or 3 times the width of pharynx. Intestinal ceca slender, extend to posterior end of body. Excretory bladder tubular, S-shaped, with tubules arising a little behind the anterior end. Testes, spherical, large, close together, in tandem in posterior fourth of body, measure 0.28 mm by 0.30–0.51 mm. Seminal vesicle tubular, extends a short distance behind acetabulum. Genital pore immediately anterior to acetabulum. Ovary, anterior to testes, oval, measures 0.15–0.23 mm by 0.13–0.16 mm. Seminal receptacle, an elongate sac, forms obtuse angle close to right of ovary, measures 0.13–0.17 mm by 0.30–0.40 mm. Mehlis' gland immediately anterior and left of ovary. Laurer's canal present. Vitellaria, extracaecal, densely follicular, extend along sides of body from level of acetabulum to level of anterior testis. Vitelline ducts, slightly anterior to ovary, meet dorsal to ovary. Uterus, long, in transverse loops packed with eggs, fills space between genital complex and acetabulum and may overlap intestinal ceca. Eggs, small, operculate with slightly thickened rim, minute boss, miracidium developed, measure 13μ by 25μ.

Host: Cormorant, *Phalacrocorax auritus.*
Habitat: Bile ducts
Locality: Illinois River, five miles northwest of Grafton, Illinois, U.S.A.
Type specimen: U. S. National Museum, Washington, D. C. No. 37071

Discussion: A study of the seventeen species already recognized in this genus reveals that all mammalian forms, i.e., *O. tenuicollis, O. felineus, O. viverrini, O. noverca, O. sinensis* and *O. tonkae* are described as lanceolate in shape, while those from fish, i.e., *O. piscicola* and *O. pedicellata,* and from a snake, *O. ophidiarum,* and from birds, i.e., *O. geminus, O. obsequens, O. longissimus, O. simulans, O. dendriticus, O. asiaticus, O. entzi* and *O. skrjabini* are all much elongated, with the exception of *O. geminus* and *O. obsequens* which are lanceolate. Thus for convenience, we might group species of *Opisthorchis* into two types: a mammalian type in which the body is lanceolate and is about three times as long as broad, and an avian and lower vertebrate type in which the length is about six times or more the body width. Our new species is of the latter group. The outstanding features of

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* Contribution from the laboratory of the Zoology-Physiology Department, University of Illinois, Urbana.

the present species are: (1) the large oral sucker as compared with the size of the body; (2) the distribution of the vitellaria from acetabulum to anterior testis continuously without interruption and laterally outside the intestinal caeca; and (3) the large round testes which occupy the last quarter of the body. All these characters make it readily distinguishable from the rest of the genus. Although in *O. pedicellata* the distribution of the vitellaria is similar, they do not extend back to the anterior testis and, moreover, they are in eight groups of follicles which overlap the intestinal caeca. Again, the presence of cuticular spines distinguishes it from *O. geminus, O. obsequens, O. longissimus, O. simulans, O. dendriticus, O. asiaticus* and *O. entzi.* The absence of a prepharynx separates it from *O. pedicellata, O. entzi* and *O. ophidiarum* and by having an oral sucker larger than the acetabulum it is easily distinguishable from *O. pisicicola.*

In regard to *O. skrjabini,* the description is so brief that there is doubt whether it belongs to *Opisthorchis* or to *Amphimerus.* The vitellaria were described as beginning in front of the acetabulum which is characteristic for the genus *Metorchis* but without statement of where they end. There is, on the other hand, no figure accompanying the text. Thus the exact generic identity of *O. skrjabini* is subjected to question. However, because of the above mentioned character, it is no doubt different from all other species in the genus *Opisthorchis.*

Opisthorchis anatis Yamaguti, 1933 and *Opisthorchis tsingkiangpuensis* Hsü and Chow, 1938

Judging from the original descriptions, drawings and photographs of *Opisthorchis anatis* Yamaguti, 1933 and *O. tsingkiangpuensis* Hsü and Chow, 1938, it is obvious that they should be transferred to the genus *Amphimerus.* In their description of *O. tsingkiangpuensis,* Hsü and Chow distinguish it from *O. anatis* by the shorter body length, lobulated testes and longer distribution of vitellaria. However, they admit that their species could be regarded as identical with *O. anatis.*

In the author's collection of helminths from Kweiyang, China, there are specimens from the livers of ducks. They correspond well with the description of *O. anatis* except the testes which instead of being rounded are slightly lobed. In his study of the variation of the shape of the gonads, Erhardt (1935) figured the testes of *O. tenuicollis* and *O. felineus* which show a great variation from round to highly lobed. Thus, at least in this genus, the shape of the testes could be used only as a supplementary character. A careful study of Hsü and Chow's Plate 1 reveals that the *O. tsingkiangpuensis* as photographed was a specimen fixed in a shrunken condition. This is especially true on the anterior half of the body at the sides of which wrinkles may be observed very clearly. If the worm were fixed extended it could have been longer, especially the anterior half of the body and might have attained the normal shape and proportions of *O. anatis.* The vitellaria in both species are confined in the posterior half of the body, although they extend a little more anteriorly in *O. tsingkiangpuensis.* This difference is very slight and by better fixation it could be eliminated. Thus it seems safe to regard *O. tsingkiangpuensis* Hsü and Chow, 1938 as a synonym of *O. anatis* Yamaguti, 1933. The latter should be removed to the genus *Amphimerus* as *A. anatis* (Yamaguti, 1933).

Syngamus hexadontus sp. nov.
(Figs. 5–13)

Specific diagnosis: worms alive, red in color with the characters of the genus; stomal papillae six, two lateral, two subdorsal, two subventral; amphids dorsal, close to lateral papillae; cervical papillae two, lateral, protrude externally near oesophageal base; excretory pore, ventral, opens midway of oesophagus near nerve ring; cuticula smooth.

Male, 7.7 mm long, 0.34 mm wide at bases of oesophagus and bursa, greatest diameter 0.44–0.45 mm midway of body, extends slightly beyond anterior end of female in permanent copula. Stoma, terminal, bowl-shaped, wall about 30 μ thick, greatest diameter at anterior third, 0.28–0.34 mm, depth, 0.19–0.27 mm including rim; cuticular rim, hexagonal, 0.038–0.047 mm high, opening circular, 0.32–0.38 mm in diameter; with six elongate, triangular teeth at base, two lateral, two subdorsal, two subventral. Spicules, equal, straight, about 90 μ long. Bursal rays, short, stumpy; ventrals, cleft, arise like laterals from common trunk; externo-dorsals well separated from dorsal, the right thicker than left, may split at base.

Female, 21.3 mm long, 0.69–0.75 mm wide at vulva. Stoma similar to that of male 0.41–0.45 mm in diameter, 0.32–0.34 mm in depth; rim, 0.43–0.45 mm in diameter, 38 μ high; wall, 36 μ thick; six triangular teeth at base of stoma, measure 50–56 μ in length. Oesophagus, 1.01 mm long, 0.20 mm wide. Vulva, oval, ventral, 6 mm from anterior end. Anteriorly, ovary extends to base of oesophagus; posteriorly, nearly to anus. Rectum, 0.14 mm long. Tail, pointed, sharp, measures 0.41–0.43 mm long. Eggs, elliptical, operculate, undeveloped when laid, measure 36–42 μ by 75–84 μ.

Host: Cormorant, *Phalacrocorax auritus.*
Habitat: Trachea.
Locality: Illinois River, five miles northwest of Grafton, Illinois, U.S.A.
Type specimen: U. S. National Museum, Washington, D. C. No. 37072

Discussion: Syngamus hexadontus sp. nov. can be readily distinguished from the rest of the avian forms of the genus by: (1) the number of teeth at the base of the stoma; (2) the dorsal ray of the male; and (3) the spicules. Its relatively large size makes it distinct from all except *S. trachea.* In *S. trachea,* and *S. parvus* the tail is blunt and in our species as well as in *S. microspiculum* it is sharply pointed. Both *S. merulae* and *S. microspiculum* do not have the anterior rim. The most closely related species is *S. microspiculum.* It is not only similar in structure but is harbored by the same kind of host. Besides the above stated characters, it also differs in the size of body, the absence of a rim at the anterior end, and its ovary being far behind the oesophagus, according to the drawings. Moreover, the characteristic eggs of the Asiatic species make it readily distinguishable.

Description of Plate

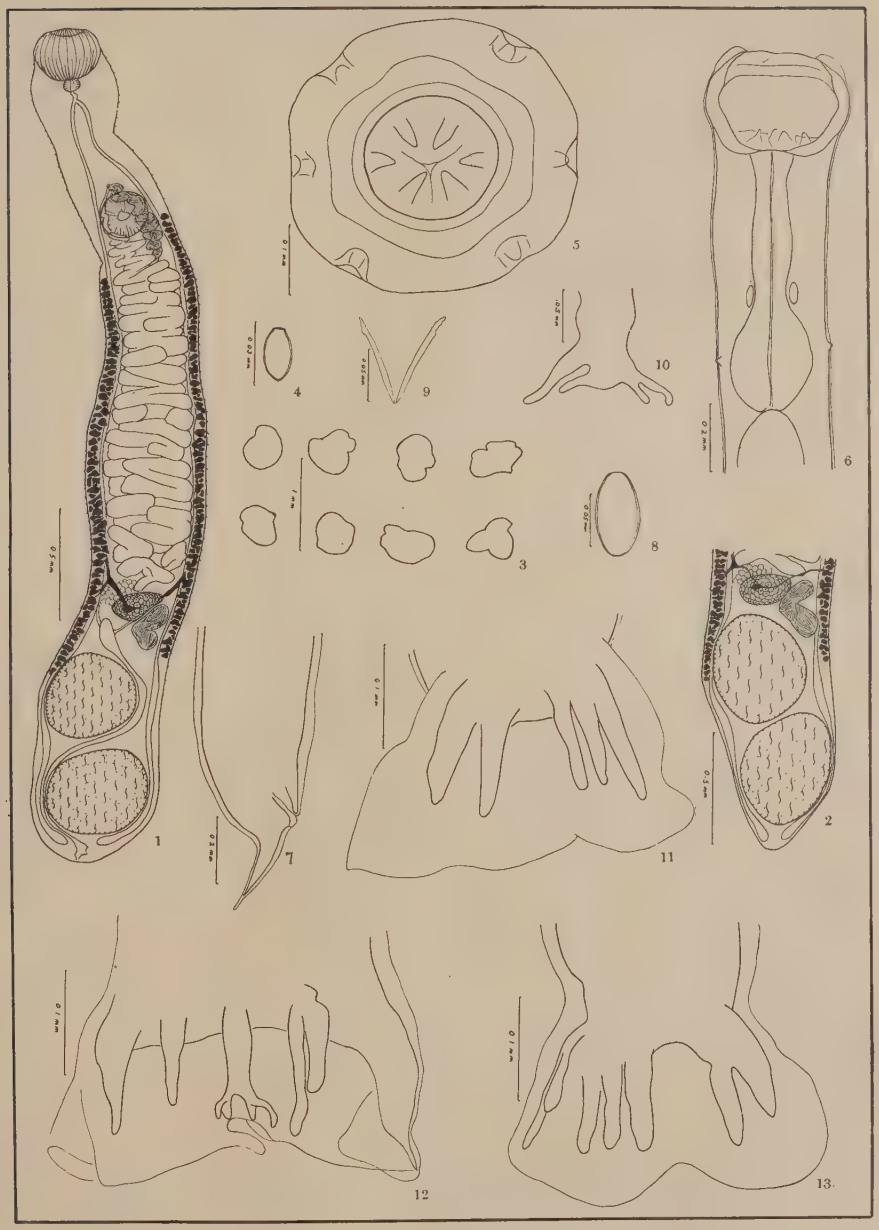
All drawings were made with the aid of a camera lucida.

Opisthorchis vitellatus sp. nov.

Fig. 1. Dorsal view of whole mount.
Fig. 2. Posterior end of body, showing variation in shape.
Fig. 3. Testes of *Opisthorchis anatis* from Kweiyang, showing variation in shape. The two at the extreme right are freehand drawings from photograph of Hsü and Chow's *Opisthorchis tsingkiangpuensis.*
Fig. 4. Egg.

Syngamus hexadontus sp. nov.

Fig. 5. Face view of anterior extremity showing arrangement of the six teeth.
Fig. 6. Dorsal view of anterior end.
Fig. 7. Posterior end of female, lateral view.
Fig. 8. Egg.
Fig. 9. Spicules.
Fig. 10. Dorsal ray showing variation.
Fig. 11. Left lobe of bursa.
Fig. 12. Dorsal lobe of bursa.
Fig. 13. Right lobe of bursa.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The presence of six teeth and the more filamentous spicules make it similar to the genus *Cyathostoma*. However, the shape of the stoma and the bursa as well as its rays make it definitely a member of the genus *Syngamus*. Thus the generic diagnosis should be emended to involve the six-toothed *S. hexadontus*, which is the only one in the genus.

The author wishes to express his gratitude to Professor Lyell J. Thomas, who directed the work and made helpful suggestions throughout the study. To Dr. H. B. Mills of the Illinois State Natural History Survey, the author is indebted for sending the birds.

SUMMARY

Two new species of helminths are described from the cormorant, *Phalacrocorax auritus* shot on the Illinois River, five miles northwest of Grafton, Illinois. One is a trematode of the genus *Opisthorchis* and the other a nematode of the genus *Syngamus*. They are named *Opisthorchis vitellatus* and *Syngamus hexadontus*. The former was from the liver and the latter from the trachea. *Opisthorchis tsingkiangpuensis* Hsü and Chow is regarded as a synonym of *O. anatis* Yamaguti and is removed to the genus *Amphimerus*.

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A NOTE ON THE OCCURRENCE OF *GONGYLONEMA PULCHRUM* MOLIN, 1857, IN MAN IN CEYLON

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The worm which forms the basis of this note was brought for identification to the Medical Research Institute, Colombo, on February 9th, 1948, by a 32-year-old Ceylonese gentleman who had extracted it from his mouth. According to this gentleman, when he had washed his mouth after dinner, he felt as if something was moving about between cheek and lower jaw of the left side. He felt a little irritation at the site, as if something was embedded in the tissues. He put his finger into his mouth and extracted a live, thread-like worm. There were no other subjective symptoms either before or after the worm was extracted.

The specimen was fixed in hot 70% alcohol and cleared in glycerine. A detailed microscopic examination has shown it to be an immature female of *Gongylonema pulchrum* Molin, 1857. It is thought that a brief description of this specimen would merit publication, as the species has not hitherto been recorded from man in India, Ceylon or the Far East. The number of cases of human infection with this parasite, in Italy (Pane 1864, Alessandrini 1914), the U.S.A. (Ward 1916, Stiles 1918 & 1921, Ransom 1923, Stiles & Baker 1928, and Waite & Gorrie 1935), the Ukraine, U.S.S.R. (Schultz & Ivanitski, 1934), New Zealand (Johnston, 1936) and Bulgaria (Sliwensky, 1941), already amounts to thirteen. This will therefore be the fourteenth case on record and the first to be reported from the East. There is nothing in the present case, however, to suggest that the infection was not one of accidental parasitism as were also those previously reported. The parasite which is known to live chiefly in the submucosa of the oesophagus of ruminants has not yet been found in local ruminants, nor has the larval form been yet recorded from any of the coprophagous insects of Ceylon.

Gongylonema pulchrum Molin, 1857
(Nematoda: Spiruridae)

Host: Man, 32-year-old male.
Site: Buccal mucosa, near left lower jaw.
Locality: Colombo, Ceylon.
Description of immature female: (Fig. 1): Length of worm 27 mm, maximum thickness 0.187 mm; diameter of mouth, when the two lips are fully extruded, 0.026 mm; anterior region covered in each submedian field by numerous well-developed cuticular bosses which extend to a distance of 1.139 mm from the anterior end; cuticular striations at intervals of about 0.007 mm; lateral cervical alae begin at a distance of 0.170 mm from anterior end; right and left cervical papillae 0.116 mm, nerve-ring 0.255 mm and excretory pore 0.408 mm from anterior end; pharynx 0.037 mm long, oesophagus 5.077 mm long; anterior muscular portion of oesophagus measures 0.425 mm; the bluntly conical tail measures 0.139 mm, and the vulva is situated ventrally at a distance of 2.040 mm from the tip of the tail. The vagina leads into the vulva from an anterior direction; the opposed uterine branches join with the vagina nearer the middle

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of the body. The eggs are not fully developed, being devoid of shells; they are mostly shapeless bodies, although quite a number are ovoid, measuring 0.020–0.034 × 0.014–0.017 mm. The state of development of the eggs and the relatively small size of the worm indicate that the specimen is an immature female of the species.

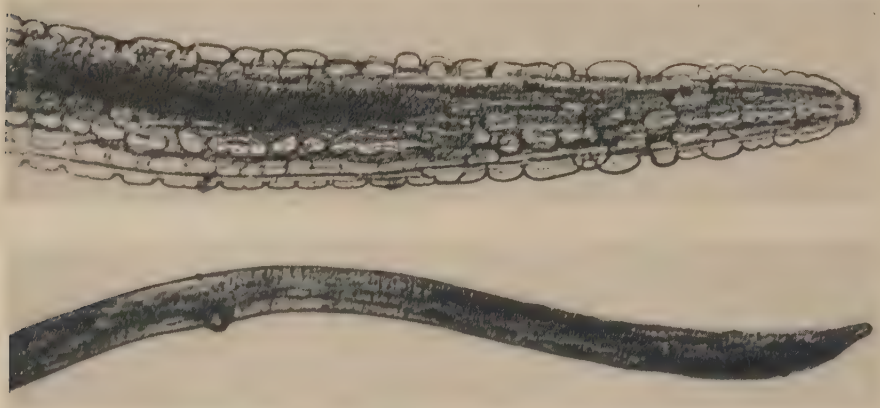

FIG. 1. *Above:* Anterior end of immature female of *Gongylonema pulchrum,* showing characteristic cuticular bosses. The anterior end of the less transparent posterior portion of the oesophagus can also be clearly seen (×146). *Below:* Posterior region of same worm, showing posterior end of vagina, vulva and bluntly conical tail. (×46).
(Photomicrographs by H. V. Claasz, University of Ceylon)

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FURTHER STUDIES OF THE EFFECT OF AGE OF MICE UPON ADULT *TRICHINELLA SPIRALIS*

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Recently several investigations relating to age resistance of mice to *Trichinella spiralis* were presented. Rappaport (1943) made a comparative study of larval development in three strains of trichinae. He used hosts varying in age from seven weeks to six months. Although he did not present individual age records, he stated that using mice of various ages within the experimental groups was permissible; because no correlation existed between age of host and the number of muscle larvae harbored. Riedel (1948) reported that young mice were more susceptible to trichina larvae than old mice. Limiting the period of parasitism to six days or less Riedel (1948) and Larsh and Hendricks (1949) reported that old mice were no more resistant to adult trichinae than young mice.

Since it takes several days for infective larvae to reach maturity and a 6-day period of parasitism may have been inadequate time to test age resistance, it was decided to study the effect of age of mice on the persistence of adult *T. spiralis* after an extended period of parasitism.

METHODS

The white mice raised under laboratory conditions were individually infected by feeding with forceps small portions of mouse diaphragm containing 100 ± 5 microscopically counted *T. spiralis* cysts. The fifteenth day after infection the mice were autopsied, the small intestines were removed, slit and dipped into clear water to free them of their food content. The presence of the food content made it difficult to count the adult trichinae, and its elimination did not cause the loss of worms embedded in the intestinal lining. The intestine of each mouse was cut into four-inch lengths and refrigerated in Petri dishes containing a solution of one percent sodium hydroxide. After the mucus had dissolved, the strips of intestine were placed on large glass slides and checked for adhering adults. The adults retained in the sodium hydroxide solution were counted directly with a stereoscopic microscope.

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental data in Table 1 definitely show that the young mice in each experiment harbored more adult trichinae than the old mice. This was not in agreement with the findings of Riedel (1948) and Larsh and Hendricks (1949) since they reported the absence of age resistance of mice to adult *T. spiralis*. That the results of the present investigation were not in agreement with those by previous investigators may be attributed to the difference in the lengths of the periods of parasitism. The former investigators allowed not more than a 6-day period of parasitism, whereas in the present study it was 15 days.

The investigations by Matoff (1936 and 1937) showed that in dogs and pigeons where age is a factor of resistance, fewer larvae matured in the old hosts. In mice, age has no eliminative influence before larval maturity. This was indicated by a

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short period of parasitism sufficiently long (five days) to permit larval maturity (Larsh and Hendricks, 1949). When the period of parasitism was extended to 15 days or beyond that required for larval maturity, as was the case in the present study, elimination was greater in the older mice.

TABLE 1.—*The numbers of adult* T. spiralis *harbored by young and old mice 15 days after infection with 100 ± 5 cysts. Age of young mice 26–32 days, age of old mice 157–171 days*

Young Mice		Old Mice	
Mouse number and sex*	Number of worms harbored	Mouse number and sex*	Number of worms harbored
	Series 1		
1M	10	1M	2
2M	9	2M	0
3M	8	3M	0
4M	14	4M	3
5F	5	5F	6
6F	11	6F	0
7F	10	7F	1
8F	9	8F	0
Total	76		12
Average	9.5		1.5
	Series 2		
1F	10	1F	1
2F	14	2F	11
3F	11	3F	1
4F	5	4F	0
5F	12	5F	0
6F	7	6F	0
7F	3	7F	0
8F	7	8F	0
9F	4	9F	1
10F	5	10F	0
11F	8	11F	0
12M	13	12M	0
13M	15	13M	0
14M	8	14M	0
15M	12	15M	0
16M	6	16M	1
17M	10	17M	0
18M	7	18M	0
19M	5	19M	0
20M	6	20M	1
21M	8	21M	0
22M	7	22M	0
Total	183		16
Average	8.3		0.7

* F—female, M—male

SUMMARY

The effect of age upon resistance of mice toward adult *Trichinella spiralis* was studied. On the fifteenth day after infection with 100 ± 5 cysts it was found that the young mice (age 26–32 days) harbored more adult trichinae than old mice (age 157–171 days).

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TWO NEW SPECIES OF TROMBICULID MITES FROM MALAYAN BATS

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Opportunity was taken, incidental to recent field studies in Malaya of mite-borne scrub typhus (Philip, Traub and Smadel, 1949), to collect a few bats in the famous Batu Caves near Kuala Lumpur. Two undescribed species of larval chiggers were collected from 2 *Eonycteris spelaea* in the fore-part of one of the caves.

Larval mites of bats in the Southwest Pacific area are of added interest because it is presumed that the genotype, *Trombicula minor* Berlesé, is a bat parasite in the larval stage since the 2 adult types were collected in guano in Tjompea Cave near Buitenzorg, Java, in 1904. Some systematic confusion on a generic level has since arisen as most recently summarized by Ewing (1944). Pending the determination of what is the real larva of the Javanese *T. minor*, it is of special concern to obtain all data possible on bat infesting species in the region. The writers are indebted to Dr. A. Diakonoff of the Zoological Museum, Buitenzorg, for attempting to re-collect the species in Tjampea Cave. Unfortunately, results thus far have been negative.

A number of species of mites have been taken on Pacific-Asiatic bats, and it is not improbable that one of them will eventually have to be synonymized as the true larva of *T. minor*. The two species described below belong to the genus *Trombicula* as presently conceived, and each has certain peculiar features.

Trombicula batui n. sp.
(Fig. 1)

Distinguished by its small size, unusually small scutum and bifurcate palpal claw (but differing from *Eutrombicula* spp. by the outer, not the inner prong being the smaller); furthermore, the forked (not branched) sensillae are submedially situated, the scutum is subquadrate and coarsely pitted; the midcoxal setae are bare as are also the three setae on palpal segment IV; and the setae on other palpal and coxal segments are forked or branched (Fig. 1).

Size of fully engorged larva not determined. Partially fed specimens show some lateral "humping" and caudal tapering as figured, 190 × 333 microns. Color pallid.

Capitulum: Chelicerae stout with the usual tricuspid cap. Cheliceral base longer than wide. Galeal setae bare.

Scutum: Exceptionally small, slightly wider than long, coarsely punctate particularly above the sensillary bases at the "eyebrows," upper corners rounded, lower corners angulate but hardly produced, the sides slightly convergent anteriorly, hind margin evenly rounded, fore-margin biconcave or sinuous, the anterolaterals situated caudad of margin, farther posterior than the anteromedian seta. Sensillae with a simple, equal branched fork at or just below the middle and their bases widely separated just under the median line. The other setae increasing in length in the order AM, AL, PL, and coarsely barbed. Standard measurements of holotype and paratypes in table I.

Dorsum: Eyes 2/2 in ocular plate. Dorsal setal counts fairly constant: 2, 8, 6–8, 6, 4–6, 4, 2; total about 36. All setae sparsely barbed. In three paratypes, the second submedian pair in row 2 are depressed out of line (as seen in *Euschöngastia indica*).

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Venter: Two pairs of sternal setae plus about 40 increasing slightly in length caudally. *Legs:* All legs with seven segments. Coxal setae, 1–1–1; those on I and III branched, that on II nude except for barely perceptible barbs basally on one specimen. No long nude setae but the usual bare spines on the apical three segments of the hind leg. Hind tarsus short and chunky.

TABLE I.—*Standard Measurements in Holotype and Paratypes of* T. batui *n. sp.*

Specimen	AW	PW	AP	SB	PSB	AM	AL	PL	S	DS
Holotype	45	54	26.5	21	22	28	23	34	44	26
Paratype 1	36	45	23.0	16	18	26	22	27	37	24
Paratype 2	36	45	23.0	17	18	27	22	27	38	25
Paratype 3	39	45	25.0	19	19	28	23	28	39	25
Paratype 4	43	51	26.0	20	21	28	26	34	40	26

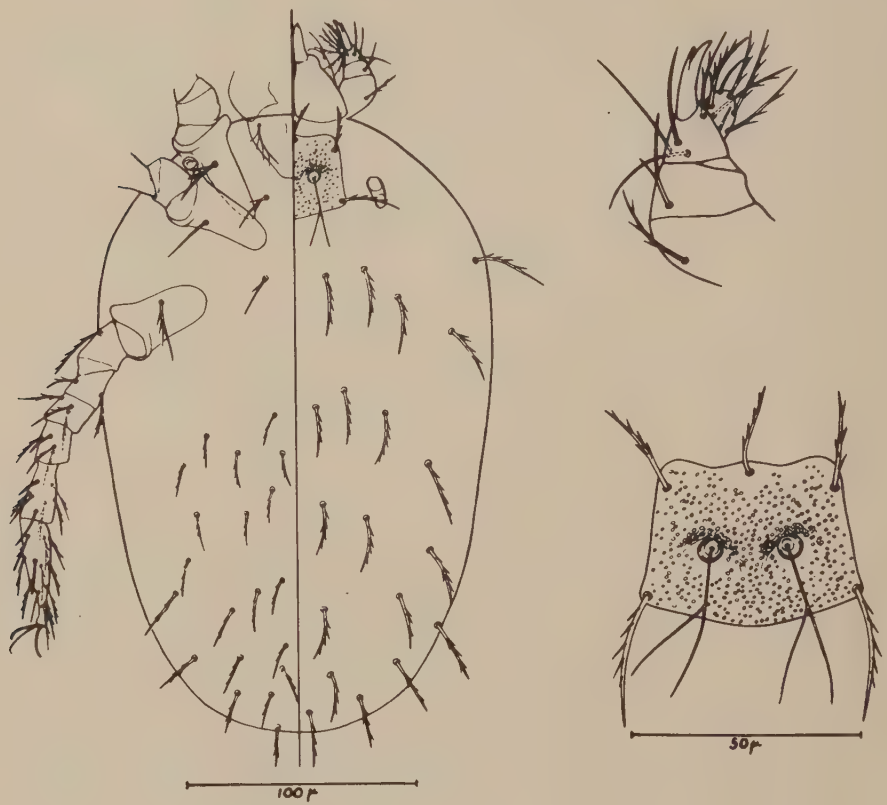

FIG 1

The unusual variation in standard measurements of this species is of interest and it is unfortunate the series is so short for checking means. The discrepancy is only apparent, as it will be noted that the proportions are the same, the figures merely representing differences in actual sizes of the specimens.

Type data: Holotype and four paratypes on separate slides, from *Eonycteris spelaea* (insectivorous bats), Batu Caves, Selangor, Malaya, 20 March 1948, No. 8011, collected by the authors (the epithet refers to the habitat rather than the host). Holotype in the United States National Museum (Type No. 1865), paratypes in

FIG 2

the British Museum of Natural History, Rocky Mountain Laboratory (AP25759) and Chicago Natural History Museum.

This species is unique among Pacific trombiculids, and its small size suggests that it may prove to be the long sought larva of *T. minor,* the adults of which are so small they were at one time considered to be nymphs. If this is *T. minor,* it would

still warrant, at most, only subgeneric separation from the bulk of species now placed in *Trombicula sens. lat.* This doubt precludes such consideration at this time, since, if the suspicion is confirmed, it would have to take the name of the typical subgenus rather than a new one.

Trombicula insolli n. sp.

(Fig. 2)

A species with a roughly trapezoidal, finely punctate scutum, the sensillae barbed on the distal 2/3 and based just above the caudal margin, the palpi with claw trifurcate and with setae on segments II to IV all bare (Fig. 2).

Body of fed larvae broadly ovoid, 536 × 286 microns; pallid.

Capitulum: Chelicerae stout with the usual tricuspid cap. Cheliceral base longer than wide. Median prong longest of the trifurcate palpal claw. Galeal setae nude. Palpal setae branched only on segments I and V (the thumb), those on II to IV nude.

Scutum: Anterior corners rounded, posterior corners slightly produced, the hind margin not extended, nearly straight across between the posterolateral setae. The latter longer than the subequal anteromedian and anterolateral setae. AM situated slightly in advance of the AL, but all are behind the anterior margin. All scutal setae barbed, the sensillae bare on their basal third. Sensillary bases situated at about the posterior fourth of the scutum. Standard measurements of holotype and paratypes in table II.

Dorsum: Eyes 2/2 in ocular plate. Dorsal setal counts somewhat variable: 2, 8–9, 8–10, 8 plus about 20. Caudal rows irregular, and setae a little shorter in length.

Venter: The usual two pairs of sternal setae plus 18–20, those posterior to the anus slightly longer.

Legs: All legs with seven segments. Coxal setae, 1–1–1, all branched. Hind leg with one nude mastifemorala on the telofemur, a mastigenuala on the genu in addition to the usual bare spine on the latter and on the tibia. Hind tarsus slender and elongate.

TABLE II.—*Standard Measurements of Holotype and Paratypes of* T. insolli *n. sp.*

Specimen	AW	PW	AP	SB	PSB	AM	AL	PL	S	DS
Holotype	52	69	36	22	12	44	42	57	68	45
Paratype 1	48	65	35	23	11	46	42	64	..	45
Paratype 2	50	65	35	23	12	45	42	60	65	45

Type data: Holotype and two paratypes on separate slides; from *Eonycteris spelaea* (insectivorous bats), Batu Caves, Selangor, Malaya, 20 March 1948, No. 8011 collected by the authors, 4 April 1948, and No. 8015, collected by R. Traub and H. Ley. Holotype in the U. S. National Museum (No. 1866), a paratype each in the Rocky Mountain Laboratory (AP25759) and British Museum.

The species is named for Mr. Ben Insoll of the Selangor Museum, who helped us immeasurably by serving as guide and collector, and who developed a second attack (additional to a pre-war attack) of scrub typhus due to field exposure during our investigations.

It was at first suspected that this species represented the re-discovery of *T. piercei* Ewing originally from a bat in the Philippine Islands. The types in the U.S. National Museum appeared to have been mounted from dried specimens, and the original description is not very informative. The five cotypes of *T. piercei* have been remounted on individual slides with some improvement, and the specimen on the slide with replaced original labels is herewith designated as the lectotype.

The palpal claw is trifurcate, the palpal setae on segments II to IV appear bare. The scutum has much the same trapezoidal shape as *T. insolli* sp. nov., with the sensillary bases near the hind border (sensillae missing), but AL and AM are in line nearly on the anterior border, and the standard data are significantly different. The

ALs are shorter than AM, and the whole scutum has larger proportions. The barbs on the DS are also shorter but the formula cannot be determined due to distortion. Table III compares the standard data of this specimen with the holotypes of the two new species.

TABLE III.—*Standard Measurements of Holotypes Compared to those of a Related Philippine Species.*

Species	AW	PW	AP	SB	PSB	AM	AL	PL	S	DS
T. batui n. sp.	45	54	26.5	21	22	28	23	34	44	26
T. insolli n. sp.	52	69	36	22	12	44	42	57	68	45
T. piercei Ewing	60	83.5	56.5	26.5	..	33	50	70	..	..

T. muscae Oud., *T. russicum* Oud., *T. schmitzi* Oud., *T. minutissimum* Oud., *T. myops* Vitz., and a species collected by one of us (C.B.P.) in New Guinea to be described by Womersley, are other bat-infesting species that differ in varying characters from the above.

SUMMARY

Described as new from Malayan bats are the larval mites: *Trombicula batui* n. sp., and *T. insolli* n. sp. (holotypes in the U. S. National Museum, Washington, D. C.). A lectotype is established from among the remounted cotypes of *T. piercei* Ewing from bats in the Philippine Islands and comparative characters discussed.

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THE ACQUISITION OF ISOTOPICALLY LABELED INORGANIC PHOSPHATE BY THE TAPEWORM, *HYMENOLEPIS DIMINUTA*, WITH SOME REMARKS ON THE HOST-PARASITE RELATIONSHIP[1]

CLARK P. READ[2]

The occurrence of phosphorus in tapeworms has been recorded by several workers. Phospholipids have been determined in *Diphyllobothrium latum* by Faust and Tallquist (1907) and Totterman and Kirk (1939). Von Brand (1929) reported that 15% of lipid material extracted from *Moniezia expansa* was phospholipid. Smorodinzew and Bebeschin (1939 a, b) quantitatively determined the lipid content of *Taenia saginata* and found 5.41% lecithin and 3.75% cephalin. Salisbury and Anderson (1939) and Lesuk and Anderson (1941) found the lipids of *Cysticercus fasciolaris* were 30% phospholipid. Shopfer (1932) found that the cystic fluid of *Cysticercus tenuicollis* contained 0.12 to 0.15 grams of phosphorus per liter. Salisbury and Anderson (1939) reported 5.37% phosphorus in the ash of *C. fasciolaris*. These scanty data suggest that phosphorus may play an important role in the physiology of these helminths. This might be expected in view of the importance of this element in the tissue chemistry of vertebrate animals. Preliminary experiments by the writer, not yet published, indicate that glycolysis in tapeworms is of the phosphorylative type. The present report is concerned with the gross uptake of phosphorus by the cestode, *Hymenolepis diminuta*, determined by the use of radioactive phosphorus (P^{32}).

MATERIALS AND METHODS

Hymenolepis diminuta was reared in three to four month old albino rats, weighing 125 to 140 grams, infected in the manner described by Chandler (1939). The animals were used for experimental purposes three to four weeks after feeding each rat ten cysticeroids. Radioactive phosphorus was administered as phosphoric acid in trace doses containing less than one microgram of phosphorus. In experiments where the dose desired was above the trace level, unlabeled sodium dihydrogen phosphate was added as carrier. In various experiments the phosphorus was administered by stomach tube or intraperitoneally. To measure the radioactivity in the host gut mucosa, the small intestine was removed, washed out thoroughly with cold Tyrode's solution, and the mucosa scraped off with a razor blade. The scrapings were then weighed, wet ashed in nitric and perchloric acids, and the radioactivity determined with a glass-walled dipping counter. The worms were removed from the host gut and rapidly washed in five changes of cold Tyrode's solution. They were then carefully blotted to remove as much adhering fluid as possible and weighed. This was followed by wet ashing in nitric and perchcloric acids, and, after making the digested material to volume, aliquots were removed to counting dishes for determination of radioactivity. Counts of worm samples were made with a thin mica window Geiger-Mueller tube manufactured by Tracerlab, Inc. Sufficient counts were taken on each sample to keep fluctuation error below 2%.

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THE UPTAKE OF ORALLY ADMINISTERED PHOSPHORUS

Experiment I

Two groups of infected rats were starved for twenty-four hours. The rats of one group were given 0.5 ml. of normal saline containing a trace dose of radiophosphorus with an activity of 18×10^4 counts per second. The rats of the second group were given 0.5 ml. of normal saline containing an identical amount of radiophosphate

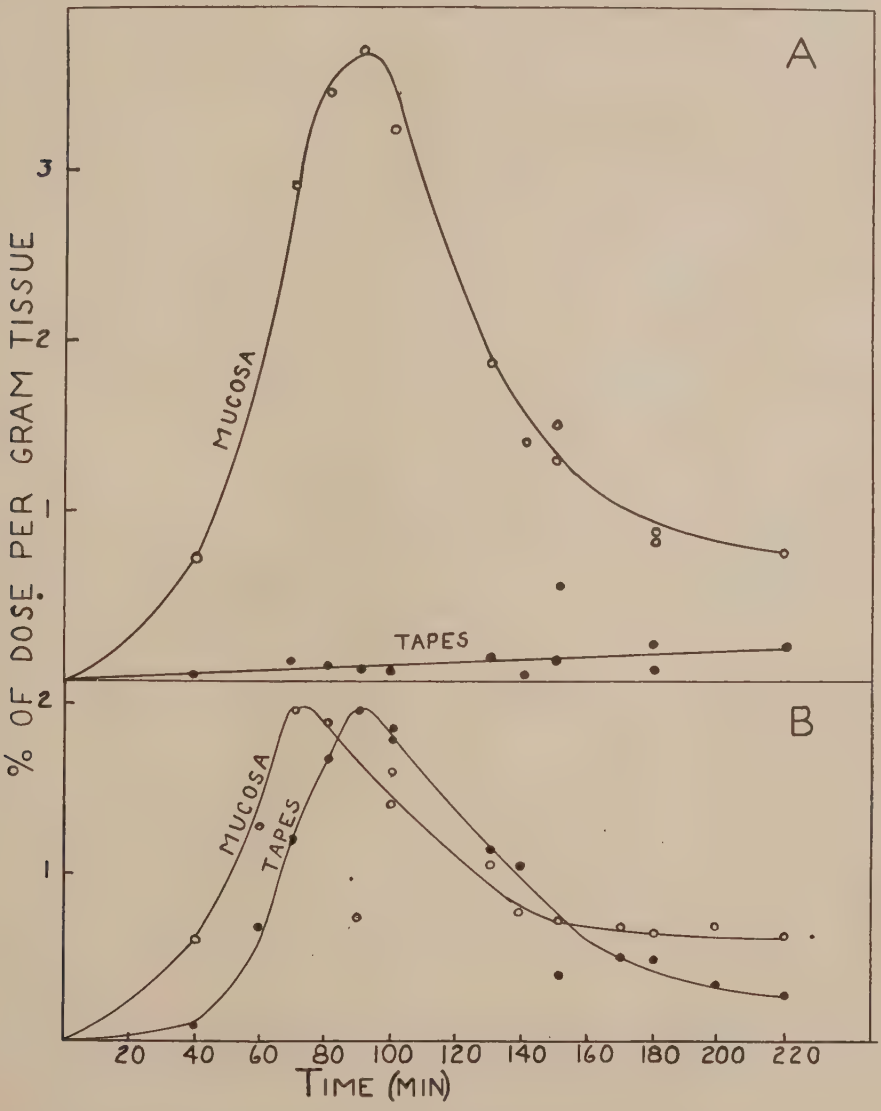

FIG. 1. The uptake of trace amounts of radiophosphate by host tissues and tapeworms when administered to the host *per orum*. A—phosphate plus glucose. B—phosphate without glucose.

and 0.5% glucose. All materials were administered by stomach tube. At intervals after dosing, the rats were killed by decapitation and the small intestine and worms rapidly removed and prepared, as previously described, for the assay of radioactivity. The results of this experiment are summarized in Figure 1. The amount of radioactive material is expressed in terms of percentage of the dose administered to the rat per gram of tissue assayed. It will be seen that in the animals receiving only phosphate the maximum activity of the host tissues is attained in about seventy minutes, while the maximum activity of the helminths is reached in eighty-five to ninety minutes. In those animals receiving phosphate plus glucose the mucosa reached a peak of activity about ninety minutes after administration of the dose. The tapeworms in these latter animals acquired the phosphate in a manner strikingly different from that seen in the animals not receiving glucose.

Two alternative explanations for this difference presented themselves. First, the glucose might exhibit some inhibitory effect on the worms to prevent the uptake of phosphate ion or cause the secretion of some inhibitory substance by the host tissues. Second, the phosphate might be taken up more rapidly in the anterior part of the small intestine in animals absorbing glucose than in those not absorbing glucose, thus making it unavailable for the worms. It was seen that the second of these explanations might be rather readily tested. Thus, an experiment was planned in which a large enough dose of phosphate was administered to insure its reaching the part of the small intestine inhabited by the worms.

Experiment II

Two groups of infected rats were starved for twenty-four hours. The rats of one group were given 5.0 mg. of labeled sodium phosphate with an activity of 18.5×10^4 counts per second in one ml. of normal saline, and the animals of the second group were given 5.0 mg. of labeled sodium phosphate and 50.0 mg. of

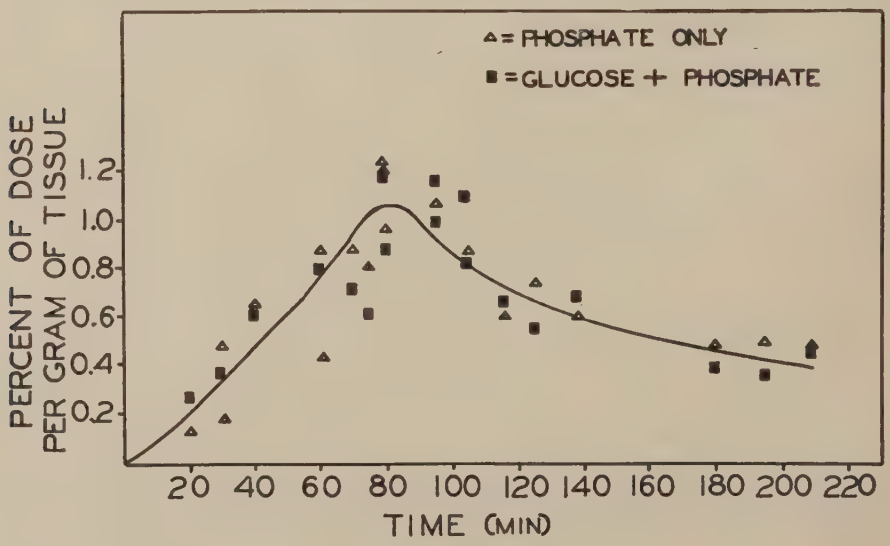

FIG. 2. The uptake of radiophosphate by tapeworm tissues when given, with or without glucose, *per orum* in macro amounts.

glucose in one ml. of normal saline by stomach tube. At intervals after dosing the rats were killed and the worms removed for assay as in Experiment I. The results are summarized in Fig. 2.

It will be seen that with larger amounts of phosphate, the uptake of phosphate by the worms is about the same in the presence or absence of glucose. It seems probable that the explanation for the results obtained in Experiment I, is comparatively simple. When trace amounts of phosphate are administered with glucose, the phosphate is absorbed from the lumen of the gut more rapidly than is the case when no glucose is given. In the former case, little or no phosphate reaches the worms which are in the lower two-thirds of the small intestine.

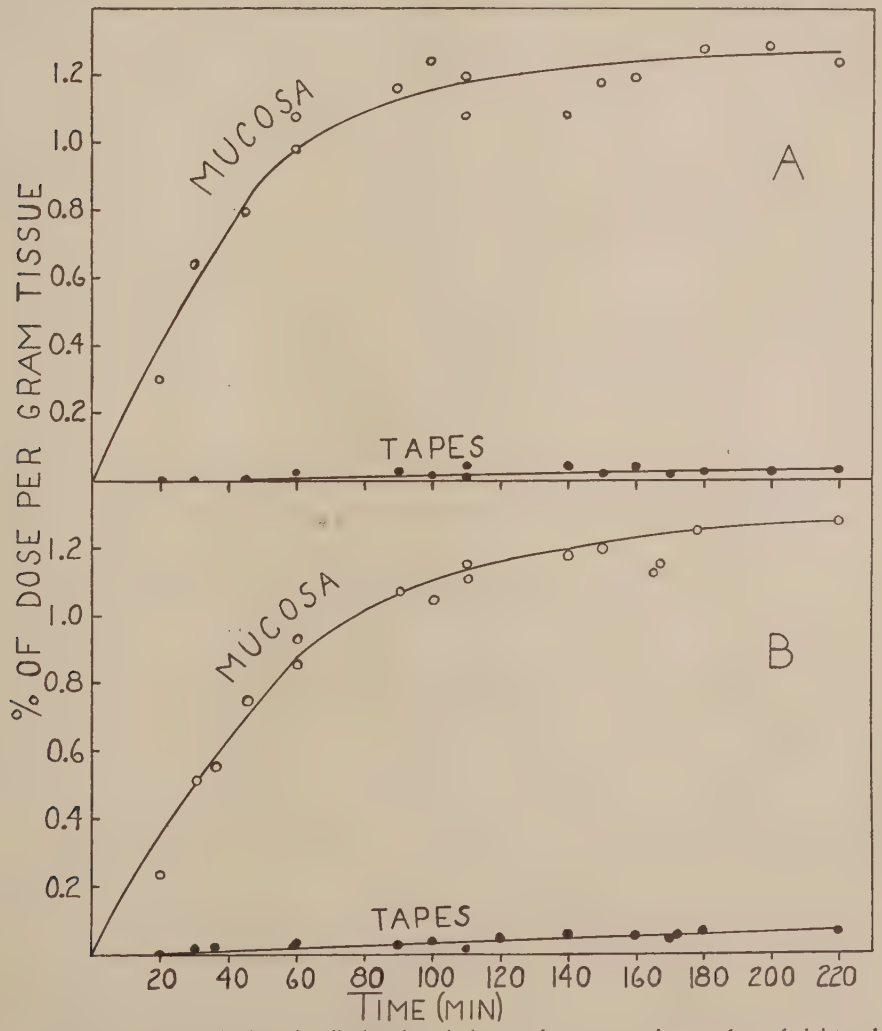

Fig. 3. The distribution of radiophosphate in host and tapeworm tissues when administered to the host by intraperitoneal injection. A—hosts given glucose before administration of phosphate. B—hosts not given glucose before administration of phosphate.

THE UPTAKE OF PARENTERALLY ADMINISTERED PHOSPHORUS

Two groups of infected rats were starved for twenty-four hours. The rats of the first group were given labeled phosphate with an activity of 18.0×10^4 counts per second in 1.0 ml. of normal saline solution intraperitoneally. The rats of the other group were given 1.0 ml. of saline containing fifty mg. of glucose by stomach tube and one hour later, an intraperitoneal injection of phosphate in saline identical with that given the rats of the first group. At intervals after dosing, the rats were killed by decapitation, and the small intestine and worms rapidly removed and prepared for assay of P^{32}. The results of this experiment are summarized in Fig. 3.

DISCUSSION

The difference in P^{32} concentration attained in the mucosa of rats absorbing phosphate with or without glucose is of some interest. This might be construed to indicate an enhanced turnover rate of phosphate in animals absorbing glucose, rather than an enhanced absorption from the lumen of the gut. However, the worms in the rats absorbing glucose do not exhibit an uptake similar to that seen in worms from hosts not absorbing glucose, but rather closely resemble the phosphate acquisition pattern of worms whose hosts have been given trace amounts of phosphate parenterally. This is evidently due to a failure of trace amounts of ingested phosphate reaching the worms when the host is absorbing glucose. The activity attained by the worms from hosts receiving phosphate plus glucose *per orum* probably represents phosphorus which is derived from endogenous sources. The writer feels that this constitutes some evidence for an enhancement by glucose of mucosal phosphate absorption. Suggestions as to the mechanism of this enhancement do not seem to be in order at this time, but it may be said that this is probably associated with the phosphorylation of carbohydrate which is thought to occur in the intestinal mucosa.

The observation that the worms in the hosts receiving phosphate by intraperitoneal injection showed a steady increase in P^{32} content with time calls attention to a rather important aspect of the relationship existing between intestinal worms and their hosts. Parasitologists have too infrequently taken into consideration the fact that the gut is a dynamic organ into which materials are constantly being secreted and from which some of these materials are reabsorbed. As a case in point, Visscher and his associates (1944 a, b) have determined the total directional rates and net transport rates of certain ions between the gut and the blood. These workers furnished strong evidence that there is a forced flow of fluid across the intestinal epithelium in both directions simultaneously, and that differences in the solute content of the water in the two streams and the relative rates of the streams determine the direction and magnitude of the net transport. Attention should be called to the significant observation of Valette and Cavier (1945 a, b) that the composition of the secretion of obstructed jejunal loop is essentially that of a transudate of blood plasma with the important exception that it is practically sugar-free, and to the report of McGee and Hastings (1945) that samples of juice from the jejunum of fasting humans are isotonic with serum. Even more significant from the parasitologists' view may be the finding of Mitchell (1926) that endogenous fecal nitrogen is more or less constant regardless of protein ingestion and is instead a function of the bulk of the diet. This has been corroborated by Schneider (1935) and other workers.

These observations may furnish some clue to an explanation of the report of Chandler (1943) that growth and maintenance of *Hymenolepis diminuta* is independent of protein in the host diet. Further, Chandler found that in rats receiving no carbohyrate in the diet, worms developed, though they were markedly stunted. The fact that any development occurred seems to the writer to be a very significant observation. It is now well recognized that carbohydrate metabolism is a predominant feature in tapeworm physiology, and the development of tapeworms in a carbohydrate-deprived host indicates that materials for carbohydrate metabolism must have been derived from endogenous sources.

It becomes increasingly apparent that attempts to gain any comprehensive knowledge of intestinal parasites must include a more complete study of this complicated organ, the vertebrate gut. Further studies with this aspect of helminth physiology in mind are being followed in this laboratory.

Appreciation is expressed to Dr. A. C. Chandler for advice and criticism in the preparation of this manuscript.

SUMMARY

1. A study has been made of the uptake, *in vivo,* of isotopically labeled (P^{32}), inorganic phosphate by *Hymenolepis diminuta.*

2. When trace doses of labeled phosphate were administered by stomach tube to starved host rats the maximum activity of host gut mucosa was reached in about seventy minutes while the maximum activity in the worm tissues was reached in eighty-five to ninety minutes. In host rats given glucose with the trace dose of phosphate, maximal mucosal activity was attained in about ninety minutes while the activity in worms from these rats increased slowly with time.

3. The uptake of radiophosphorus by worms from hosts given five mg. of labeled sodium phosphate (by stomach tube), either with or without glucose, was found to be very similar.

4. The uptake of parenterally administered labeled phosphorus by worms in either starved or post-absorptive host rats was similar, showing a steady increase in P^{32} content with time.

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AN ISOLATION CHAMBER FOR THE STUDY OF INDIVIDUAL ECTOPARASITES ON THEIR HOSTS

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In order to study certain phases of the life cycle of the snake mite, *Ophionyssus natricis* (Gervais), it was found necessary to devise some method of isolating individual mites under as nearly natural conditions as possible. Several artificial media, including liquid snake blood and snake blood agar, were tried without success, as the parasites either failed to feed or became bogged down in the media. Another solution was to find some method of isolating the parasites on the natural host in a manner that would permit observation of them.

Several methods, employing various designs of celluloid cells, were tried with varying degrees of success and failure. In devising a workable chamber, three main difficulties were encountered. Either the snake host or the mites were killed or, if neither were harmed, the mites were able to escape from the cell. Finally, after many unsuccessful attempts, a chamber, which retained the parasites without harming them or their hosts, was perfected.

Celluloid sheeting, celluloid tubing, microscope slide cover glasses, and acetone were the only materials used in the construction of the isolation chamber. A base (Fig. 1a), 12 mm. square, was cut from a sheet of celluloid; a hole, 7 mm. in diameter, was punched in the center with a paper punch; and four small holes, one in each corner, were drilled with a half-round surgical needle. A ring, 5 mm. in length, was then sliced from a celluloid tube whose inside diameter was 7 mm. and whose outside diameter was 8 mm. A small rectangular piece was cut from this ring with a razor blade and the cut edges were held together with a forceps and sealed with acetone. This chamber ring now had an outside diameter of 7 mm. or the same diameter as that of the hole punched in the base. The ring was then inserted into the hole in the base so that 1½ mm. extended below the base and 3 mm. extended above the base. The two parts were then fused with a drop of acetone, forming the isolation chamber (Fig. 1b).

A cap to fit over the chamber to prevent the escape of the mites and still permit observation into the cell was constructed in the following manner. Another ring, 3 mm. in length, was sliced from the celluloid tube. This cap ring had an inside diameter equal to the outside diameter of the chamber ring. Two discs, 10 mm. in diameter, were cut from sheet celluloid; and holes, 7 mm. in diameter, were punched in the center of each. A piece, 7 mm. square, was then cut from a microscope slide cover glass with a diamond point glass marking pencil. This was sandwiched in between the two discs so that the holes in the centers of the discs coincided and the cover glass completely covered the holes. These were held in place with a forceps and a drop of acetone was placed so that it would flow between the discs and seal

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them together with the glass window in between (Fig. 2a). The window was then fused to the cap ring with acetone, forming the completed cap (Fig. 2b), and the chamber was ready to be attached to the host animal.

After restraining a snake on a board designed for that purpose, the chamber was placed in the desired position on the body of the host. A half-round surgical needle with cutting edges was used to draw short lengths of silk thread through the skin of

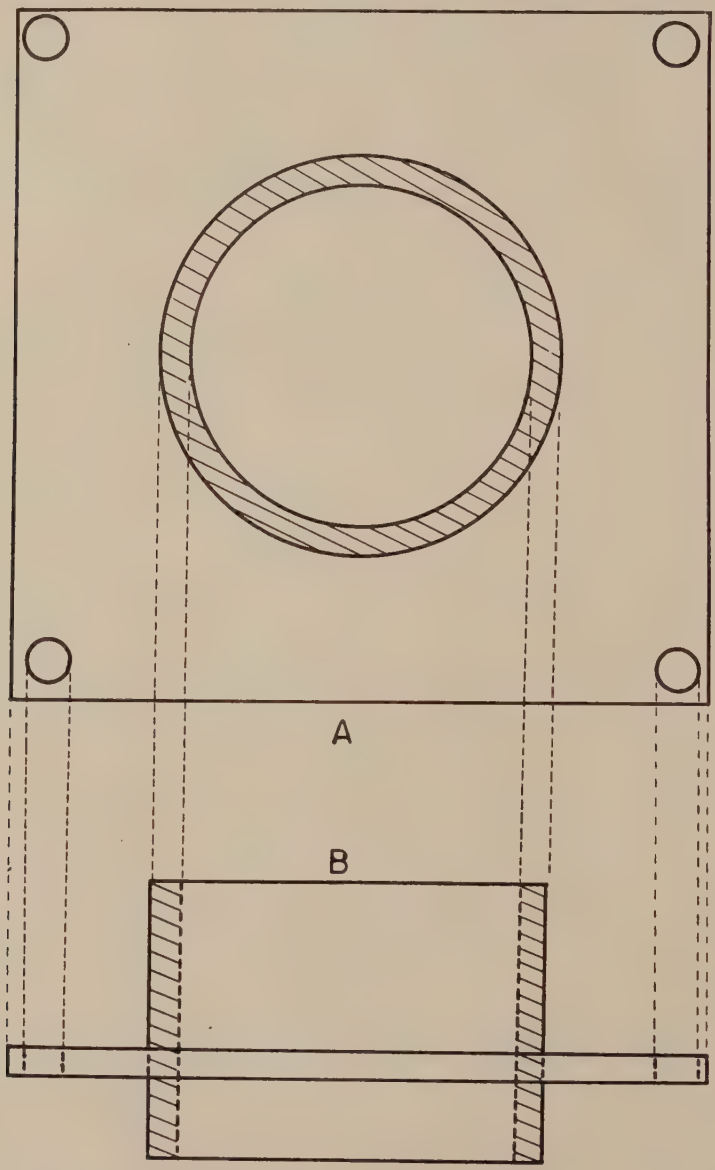

Figure 1. Isolation Chamber

the host animal and through the holes in the corners of the base. The thread was tied tightly, thereby fastening the chamber securely to the skin of the snake. The space between the base of the cell and the body of the host was filled in with "Tri-Tix" rubber cream glue, produced by Tri-Tix, Inc. of Milwaukee, Wisconsin, and

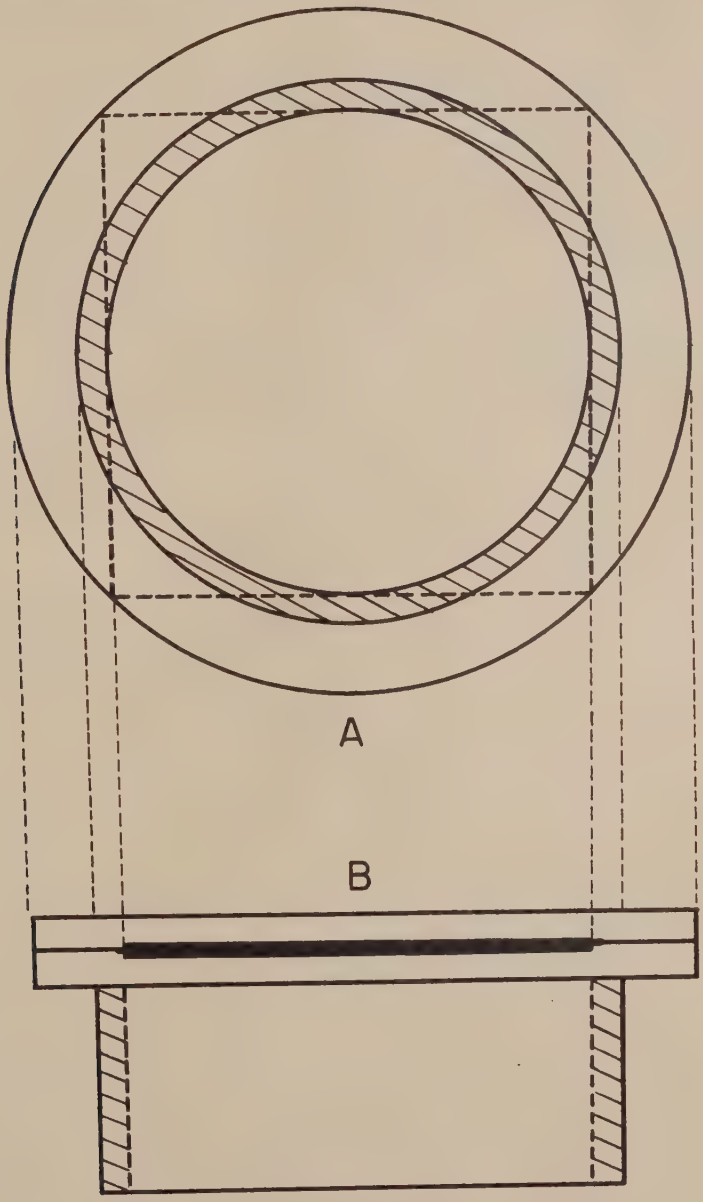

Figure 2. Chamber Cap

the snake was removed from the restraining board and placed in its cage until the cement dried. After four to eight hours the rubber cement hardens sufficiently to form an elastic seal that will prevent the escape of the parasites. Any cement that has overflowed into the chamber is easily removed with a forceps at this time. A mite was then placed into the cell, the cap was set in place, and the set-up was ready for observation.

With this apparatus it is possible to isolate individual parasites on the host and observe them throughout their development. In this manner many aspects of the life history, such as oviposition, feeding, moulting, parthenogenesis, etc., can be checked in detail. A trial chamber was fastened onto a living snake and kept in position for more than two months. During this time five generations of mites were successfully reared, spending their entire lifetime, from egg to adult, within the cell. The chamber cap was removed for approximately five minutes at intervals of about two weeks in order to remove enough mites to prevent overcrowding. At the present time, the writer is using ten of these cells on a single snake. Mites are being successfully reared in each and the host exhibits no apparent ill effects.

Although this isolation chamber was devised for and tested only in the study of the snake mite on its snake host, it is believed that it could be employed effectively for the study of many other ectoparasites on various types of host animals.

EFFECT OF X-RADIATION ON *TRYPANOSOMA CRUZI**

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The efficiency of a radioactive isotope in the treatment of a parasitic disease will obviously depend upon the radio-sensitivity of the parasite. Interest in this possibility in trypanosomiasis cruzi (Chagas' disease) led to the experiments reported here.

Halberstaedter (1914) found that *T. brucei* treated with radium (Beta-rays) lost their infectivity although they remained viable as evidenced by their continued motility. Bruynoghe (1926) confirmed these results with *T. brucei* using radium. Patel (1936) irradiated cultures of *T. brucei* with X-rays and ultra-violet light and found the organisms resistant to the former but rapidly destroyed by the latter. Halberstaedter (1938) exposed *T. gambiense* to varying dosages of X-rays and found that it took 600,000r to kill the organisms although they could be rendered non-infectious for mice by doses of 12,000r.

The present paper reports the results of experiments exposing *T. cruzi* to X-rays.

MATERIALS AND METHODS

Virulent cultures of *T. cruzi* in N.N.N. medium in Pyrex test tubes, (5¾″ long × ¾″ diam. × 1 mm. thick) were irradiated by X-rays on a 180 K.V. machine using two tubes, unfiltered, at 14.8 cm. With this apparatus 10,000r could be delivered in approximately 5 minutes. After varying exposures, 0.5 cc. amounts of the cultures were injected immediately (intra-peritoneally) into white mice weighing approximately 10 gms. Non-irradiated cultures were injected into control animals. Cultures were examined immediately before and after irradiation for viability and approximate numbers. All cultures were kept at room temperature in a dark closet. Altogether, some 100 experimental animals and 100 control animals were inoculated. Infection was considered to have occurred when the peripheral blood of the animals became positive for trypanosomes.

RESULTS

With X-ray dosages varying from 10,000r to 100,000r, the trypanosomes remained viable (as determined by motility) and morphologically unchanged in both fresh and in fixed and stained preparations. However, starting with dosages of 10,000r a definite decrease in infective power of the organisms was induced as indicated by a lengthening of the time in which the experimental animals became infected as compared to the controls. The control animals invariably became positive from the 4th to the 7th day after inoculation. The earliest infection in an experimental animal occurred on the 5th day, but the majority of the animals in any group did not become positive until several days later.

From each 100,000r irradiated culture, sub-cultures were made *on the day of irradiation* and these were allowed to grow for one week. They were then checked

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for motility and approximate numbers and 0.5 cc. amounts were injected into mice. The viability and morphology of these organisms showed no significant difference from the sub-cultures made from control cultures handled the same way. The approximate numbers of organisms, however, were somewhat decreased. None of the animals inoculated with these sub-cultures ever became infected. Though viability of the organisms was apparently unchanged, they had not recovered their virulence in one week after transfer to a new culture.

Giemsa-stained spleen smears were made from several of the experimental animals of the 100,000r group, and examined for leishmania forms, but none was ever found. To assess the possibility that a chronic, low-grade, sub-clinical infection had been induced by the irradiated trypanosomes, heart blood, spleen and liver extracts from several experimental animals of the 100,000r group were inoculated into other animals. None of these animals became infected. It was concluded that doses of 100,000r delivered to cultures of *Trypanosoma cruzi* had destroyed their infectivity, but not their viability.

TABLE I.—*Effect of Varying X-ray Dosages On Virulence of* Trypanosoma cruzi

Days after Inoculation	Control Animals	Exp. Animals 10,000r	Exp. Animals 15,000r	Exp. Animals 20,000r	Exp. Animals 50,000r	Exp. Animals 100,000r
4	*					
5		*				
6						
7	+					
8						
9						
10						
11		+				
12			*			
13			+	*		
14						
15					*	
16						
18					+	
20						
30						
40						
	* Earliest Positive + *All* Animals Positive					Total of 60 animals failed to develop infection

Both irradiated (100,000r) and control cultures were examined at intervals up to 2 months. Cultures which had remained uncontaminated appeared still to be thriving, their motility and approximate numbers unchanged. Four of the original irradiated cultures (100,000r) respectively 5, 6, 7 and 8 weeks old, were injected into mice. All of these animals became infected within 7 days, demonstrating that the cultures originally rendered non-infectious by irradiation had regained their virulence within 5 weeks.

One irradiated culture (100,000r) one month old showed only rare organisms at that time. Their morphology and viability (motility) however, were normal. From this original culture a sub-culture was made and allowed to grow one week at which time the numbers of organisms had increased and all were viable. This sub-culture when inoculated into two white mice caused infection in both.

DISCUSSION

In general, the radio-sensitivity of *Trypanosoma cruzi* here reported parallels that of *T. gambiense* and *T. brucei* as reported by others. But, while Halberstaedter found that doses of 12,000r rendered his trypanosomes (*T. gambiense*) non-infec-

tions we found that dosages somewhere between 51,000r and 100,000r are necessary to destroy the infectivity of *Trypanosoma cruzi*. Whether this is a true species difference, a strain difference, or merely an experimental difference cannot be stated, as our materials and methods were different.

It was observed that uncontaminated irradiated cultures (100,000r) appeared to be thriving up to 3 months after irradiation suggesting that neither viability nor reproductive power were affected although infectivity was. Also, the observation that cultures made from irradiated (100,000r) cultures on the day of irradiation and allowed to grow for one week were non-infectious for mice, whereas the original irradiated cultures regained their infectivity after a month or more suggests that the cultures had renewed themselves with new generations of trypanosomes to which the effects of radiation had not been passed.

ACKNOWLEDGMENTS

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CULTURAL AND PHYSIOLOGICAL OBSERVATIONS ON *TRYPANOSOMA RHODESIENSE* AND *TRYPANOSOMA GAMBIENSE*

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The cultivation of the trypanosomes of the *brucei* group is generally conceded to be much more difficult than that of the members of the *lewisi* group. While the latter have been cultivated in a variety of media and have been studied to some extent from the standpoint of their nutritional requirements and metabolic activities (Lwoff, 1940; von Brand, Johnson, and Rees, 1946; Chang, 1948), the cultivation of the former has been less successful, and therefore very little physiological information is available concerning them. Reichenow (1937b) stated that the addition of sugar did not improve his medium for the cultivation of the pathogenic trypanosomes. von Brand and Johnson (1947) found that the respiration of the proventricular form of *T. gambiense* was sensitive to cyanide. This is in contrast to that of its blood-stream form.

Three types of media have given some measure of success in the cultivation of the pathogenic African trypanosomes. On blood agar media they grow either in the water of condensation (Novy and McNeal, 1904; Thomson and Sinton, 1912), or form colonies on the surface of the agar (Weinman, 1946). In liquid media (von Razgha, 1929; Reichenow, 1932, 1934; Brutsaert and Henrard, 1938) they aggregate commonly on the surface of the settled red cells. Slightly more viscous media were developed by Ponselle (1924) and Weinman (1944). They did not indicate where growth occurs in this type of medium.

Most investigators use human blood as the blood of choice in the preparation of their media. Those who have used animal blood (Thomson and Sinton, 1912; Reichenow, 1932; Ponselle, 1924; Prates, 1928) did not present data which indicate whether such blood will sustain subcultivation for an indefinite period.

In the first section of this paper we describe a diphasic blood agar medium which incorporates a number of features from the above media. It is less difficult to prepare than Weinman's (1946) medium which it resembles in several respects.

The remarkable intensity of sugar consumption by the blood-stream form of the pathogenic African trypanosomes (Yorke, Adams, and Murgatroyd, 1929; von Brand, 1933; Chen and Geiling, 1945; von Brand and Tobie, 1948) suggested the desirability of quantitative studies on sugar utilization by culture-stages of these species. The data concerning this point, as well as data on the ammonia production, are summarized in the second section of this paper.

1. Cultural observations

Media employed. Our main experiments were conducted with a diphasic blood agar medium prepared as follows:

a. *Solid phase:* Dissolve 1.5 gm. Bacto-beef (Difco); 2.5 gm. Bacto-peptone (Difco); 4.0 gm. sodium chloride; and 7.5 gm. Bacto-agar (Difco) in 500 ml. distilled water. Adjust the pH to 7.2–7.4 with NaOH and autoclave at 15 lbs. pressure

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for 20 minutes. Cool this mixture until it can be comfortably held in the hand (about 45° C.), then add whole rabbit blood, which has been inactivated at 56° C. for 30 minutes, in the proportion of 25 ml. blood to 75 ml. base. Coagulation of the whole blood is prevented by using 0.5 percent sterile sodium citrate.

b. *Liquid phase:* Sterile Locke's solution of the following composition: NaCl, 8 gm.; KCl, 0.2 gm.; $CaCl_2$, 0.2 gm.; KH_2PO_4, 0.3 gm.; dextrose, 2.5 gm.; and distilled water, 1,000 ml. is used.

c. The base is dispensed in amounts of 5 ml. or 25 ml. into test tubes or flasks respectively. The test tubes are kept in a slanted position and the flasks upright until the base has solidified. Then the liquid phase is added in amounts of 2 ml. and 10 to 15 ml. respectively. The tubes and flasks are closed with cotton plugs which need not be capped since subcultures must be made before evaporation becomes serious.

Human blood instead of rabbit blood can be used. Of two donors we found the blood of donor No. 1 superior to that of donor No. 2. Such variations in the suitability of human blood are in accord with the observations of Reichenow (1937a) and Lwoff and Ceccaldi (1939).

The diphasic medium as described above was used for stock cultures and for certain experimental studies. All cultures were incubated at 24°–25° C. The peak of population was usually reached in 10 to 14 days. The average population density in 59 flasks was 21 $\pm$ 0.84 million per ml. overlay, with 12 and 32 million representing the extremes. The flagellates grew dispersed throughout the liquid phase, and correspond to the proventricular form.

For the physiological studies described in part 2 of this paper, a liquid-medium was used, which was prepared by drawing off the overlay of the above medium after it was left in contact with the base for 6 days. The peak of population in the liquid medium occurred in 8 to 12 days and was 14.5 million per ml., with an average of about 9 million per ml.

The diphasic medium prepared as a dialysate medium according to the procedure of Tobie and Rees (1948) did not support growth of *T. gambiense* or *T. rhodesiense.* A negative result was also obtained with another diphasic blood-agar medium (Johnson, 1947) which is used in our Laboratory for the cultivation of *T. conorhini,* other members of the *lewisi* group, and the leishmanias. Some isolations were attempted using Reichenow's (1934) medium. They will be mentioned in the following section.

Observations on T. rhodesiense. The Wellcome CT strain was isolated from a man at Tinde, Tanganyika in October 1934. It was transferred cyclically to rats which were transported to London in December 1946. From there the strain was received in this Laboratory in February 1947. In March 1947 it was isolated in culture by adding a few drops of heart blood from an infected rat to our diphasic medium prepared with human blood, with no liquid phase added. Attempts to subculture the trypanosomes at the end of the first and the second week were unsuccessful. However, positive results were obtained when subcultures were made from the original tubes after an interval of 3 to 4 weeks. Further subculturing every 1 to 2 weeks did not present any difficulties. The strain has been maintained under continuous cultivation, through more than 50 transfers, over a period of more than 2 years, and shows no signs of weakening. On the seventh serial transplant rabbit blood was substituted for human blood in the same medium except that the liquid

phase was added. The shift to 2 ml. of overlay was made gradually by slight increases in each succeeding transfer.

A second attempt to isolate the strain from rats in February 1949 was not successful despite the fact that our diphasic medium containing rabbit blood, diphasic medium prepared with the blood from human donors No. 1 and No. 2, and Reichenow's medium prepared with blood from human donor No. 2, were tried. Using the same media we could not establish cultures of the Kahama strain of *T. rhodesiense,* an old laboratory strain which has been maintained in animals. These experiences bear out Reichenow's (1934, 1937a,b) view that syringe passage strains lose their ability to develop in culture rather rapidly.

Observations on T. gambiense. The Liberia strain was isolated from a native Liberian at Ganta, Liberia in July 1948. Guinea-pigs were inoculated with material obtained from a cervical gland. From these guinea-pigs, rats were infected and sent to us. The strain was established *in vitro* in November 1948, 4 months after it had been transferred to laboratory animals, in the diphasic medium prepared with rabbit blood. Just as in the case of *T. rhodesiense* attempts to establish subcultures were successful only after the flagellates had grown for at least 3 weeks in the original tubes. Since then no difficulties in subculturing have been experienced and the strain is being maintained in this medium.

An attempt to isolate the same strain from rats in January 1949 in the same medium failed. In April 1949 it was possible to isolate the strain in the diphasic medium prepared with the blood of human donor No. 1. Subcultures were established and a transfer to the regular medium containing rabbit blood was successful. The strain was isolated at the same time in Reichenow's medium, but growth was less luxuriant than in the diphasic medium.

The old animal strains of *T. gambiense,* (Wellcome TS, and Sandground's A and Rosey) failed to develop in any of the above media. They survived for a few days but it was impossible to establish subcultures.

Discussion: The chief advantages of the diphasic rabbit blood medium lie in the simplicity of its preparation and the fact that it requires no human blood, which is often difficult to procure particularly if large amounts are required. Furthermore, the flagellates develop in large numbers. Weinman (1946) stated that the yield from one flask of his medium was about 10,000,000 to 15,000,000 flagellates. With our medium the average yield from one flask was about 250,000,000. As in all hitherto developed media the same limitation exists, namely, these cultures are non-infective, the parasites developing only to the proventricular stage. Whether our medium is inferior or superior to Brutsaert and Henrard's (1938) modification of Reichenow's medium for practical diagnostic purposes will have to be decided by field work. Some of the observations described above may indicate that for primary isolation the diphasic medium made up with blood from a suitable human donor may be preferable to the rabbit blood medium.

2. Physiological observations

Technique. T. gambiense, Liberia strain, and *T. rhodesiense,* Wellcome CT strain, were grown in the liquid medium described in the previous section, with or without addition of glucose. Small flasks containing 20 ml. of the medium were closed with rubber stoppers in order to avoid evaporation. Measurements of reduc-

ing power after fermentation with yeast showed that this medium does not contain nonfermentable reducing substances. These results were in contrast to those obtained with the medium used in this Laboratory for the cultivation of *T. cruzi* (von Brand, Tobie, Kissling, and Adams, 1949).

Duplicate sugar determinations by means of Hagedorn and Jensen's (1923) technique were conducted after inoculation of the flasks and at about weekly intervals thereafter until the organisms had died. At the same periods, pH determinations were made with a Beckman pH meter, and counts of the organisms were made in a Levy counting chamber.

Ammonia determinations were made by aerating a 7 to 9 ml. sample previously alkalinized with sodium carbonate into M/10 H_2SO_4 and titrating the latter with M/10 NaOH, de Wesselow's mixture of methylene blue and methyl red serving as indicator.

Results: With *T. rhodesiense* the sugar content was determined at specified intervals in two series of 6 flasks each, appropriate samples being withdrawn aseptically every time from each flask (fig. 1). In Series A the initial sugar content was raised to about 210 mg. percent by the addition of glucose. No sugar was added to

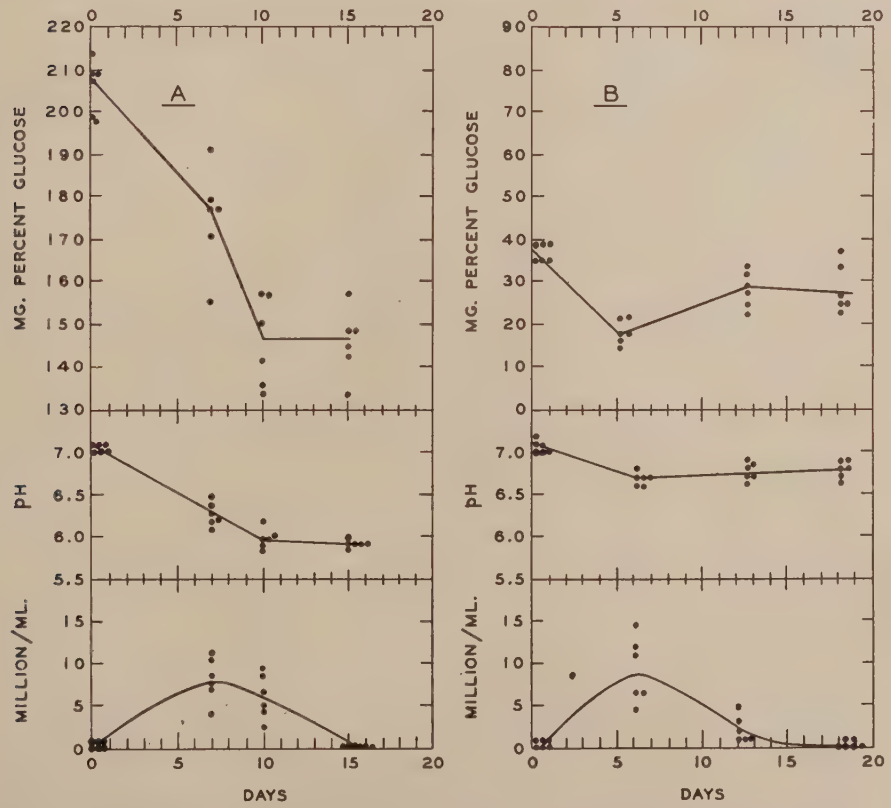

FIG. 1. Glucose, pH, and population in cultures of *T. rhodesiense* with high (Series A) and low (Series B) initial glucose content in the medium.

Series B; in this case the initial value due to the ingredients of the medium alone was only 38 mg. percent. A pronounced sugar consumption took place in Series A and the pH fell from 7.1 to 5.9. On the other hand, in Series B only about 10 mg. percent glucose disappeared and the pH dropped only to 6.7. The population reached similar values in both series.

For the study of ammonia production another procedure had to be followed because most of the contents of a flask were required for the ammonia determination. A number of flasks was therefore set up for a given series and at specified intervals one or two flasks were analyzed. Media with and without added sugar were used. The medium initially contained no ammonia. A detailed presentation of the data is unnecessary because no appreciable amounts of ammonia developed in these cultures, even after the organism had died. Only in one instance was a value of 4 mg. percent obtained.

In the case of *T. gambiense,* the sugar and ammonia determinations were made on the same samples. Since each flask served for only one set of determinations the data are less extensive than those for *T. rhodesiense.* However, they do show a very similar picture. In one series with an initial sugar concentration of 156 mg. percent the sugar concentration fell to 57 mg. percent after one week and the pH from 7.2 to 5.5, while the maximal count reached in this series was 7,000,000 flagellates per ml. Only small traces of ammonia, too low for accurate quantitative determinations, developed. The results of a second series are summarized in table 1. Just as in the

TABLE 1.—*Sugar consumption and ammonia production in cultures of* T. gambiense

Age of culture Days	Number of living flagellates per ml.	pH	Sugar mg. percent	Ammonia mg. percent
0	500,000	7.3	37	0
7	14,000,000	7.0	15	4
12	1,500,000	7.0	15	5
19	None	6.8	22	7

case of *T. rhodesiense,* it is obvious that small amounts of sugar were consumed. Small concentrations of ammonia were also observed, but it is questionable whether its accumulation was not due to autolysis of the organisms.

Discussion: The experiments reported above have shown that the proventricular forms of *T. rhodesiense* and *T. gambiense* utilize sugar if it is available and can do so even if it is present at very low concentrations. The sugar utilization is accompanied by a drop in pH, indicating the production of acids. It is clear, therefore, that these forms like all previously studied trypanosomes are aerobic fermenters. However, the concentration of sugar had no influence on the density which the population reached. It certainly was not the limiting factor. This is in accord with Reichenow's (1937b) observation and represents a marked contrast to *T. cruzi* (von Brand, Tobie, Kissling, and Adams, 1949).

The fact that in cultures with very low initial glucose content the population reached the same level as in cultures in which the flagellates consumed 100 mg. percent glucose, combined with the fact that the ammonia production was very low in both types of cultures, seems to indicate that the organisms do not utilize protein primarily by deamination. This is in contrast to the observations made by Salle and Schmidt (1928) on *Leishmania tropica.* They found a marked accumulation of ammonia in sugar-free cultures. In unpublished experiments by the present authors, similar observations were made on *T. cruzi.*

SUMMARY

1. A diphasic medium of simple preparation is described for the indefinite cultivation of *T. rhodesiense* and *T. gambiense*.

2. The chief advantage of the medium is that it contains rabbit blood and thus obviates the necessity of using human blood.

3. The flagellates develop only to the proventricular stage; hence the cultures are noninfective.

4. The proventricular forms of both *T. rhodesiense* and *T. gambiense* consume sugar with the concomitant formation of acid. They are aerobic fermenters.

5. Very little, if any, ammonia is produced by the living parasites.

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THE GENUS *OPHIOVALIPORA* HSÜ, 1935, (CESTODA: DILEPIDIDAE) WITH THE DESCRIPTION OF *OPHIOVALIPORA MINUTA* SP. NOV. FROM THE GREEN HERON (*BUTORIDES VIRESCENS* L.)[1]

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A green heron (*Butorides virescens* L.), collected near Lafayette, Indiana, in September, 1947, was found to harbor two species of cestodes. Two large individuals were identified as *Hymenolepis ardea* Fuhrmann, 1906, and over two hundred very small specimens proved to be a new species for which the name *Ophiovalipora minuta sp. nov.* is proposed. This cestode was found to be abundant in additional birds examined later. The present paper is concerned with a description of the species and discussion of its generic status.

Ophiovalipora minuta is very similar to a cestode described by Linton (1927) and identified by him as *Dilepis unilateralis* Rudolphi, 1819. This species received little further attention until Olsen (1937b) had occasion to compare it with one he described as *Dendrouterina nycticoracis,* from the black-crowned night heron, *Nycticorax n. hoactli* (Gmelin). Because of the similarity of the two species, Olsen restudied Linton's material and allocated it to the genus *Dendrouterina,* renaming the species *D. lintoni.*

Rausch (1948) described *Dendrouterina botauri* from bitterns, (*Ixobrychus e. exillis* Gmelin and *Botaurus l. lentiginosus* Montagu). He based the generic allocation of this species on a personal communication from J. G. Baer who re-examined the type, *D. herodiae,* and found that Fuhrmann was in error in regard to the disposition of the genital ducts. With the emendation of the genus, Rausch excluded the two species named by Olsen and implied that they belonged to the genus *Dilepis.* To assign the present species and the two described by Olsen (1937 a and b) to the genus *Dilepis,* as suggested by Rausch (1948), is not admissible because they clearly differ from that genus in respect to the path of the reproductive ducts and the disposition of the excretory tubules on the aporal side.

On the other hand, these species are in exact agreement with the genus *Ophiovalipora* erected by Hsü (1935) to include the single species, *O. houdemeri,* from *Elaphe carinata.* Although this species was found in a snake, Hsü stated that the natural host might be a bird since related species are parasites of homiothermal vertebrates.

A total of six green herons was examined immediately after collection to obtain parasites in the best possible condition. All were infected, the number of cestodes recovered ranging from about 25 to over 200 per bird.

It was found best to scrape the mucosa from the intestine since other methods of removing worms yielded few with the scolex intact. The tendency of the scolex to separate from the strobila complicated relaxation by shaking, and the small size made it impossible to relax them by manipulation. Procaine was not satisfactory as a

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[1] This study was made under the direction of Professor R. M. Cable. The author is indebted to Dr. Robert Rausch, U. S. Public Health Service, for providing specimens collected in Ohio and Michigan and to Dr. E. W. Price, for the loan of type material.

narcotizing agent. However, chilling of large numbers and immediate fixation yielded several relaxed specimens. The usual fixatives and staining technics were employed for whole mounts and sections.

The morphology of the egg was readily determined from living material but embryonal structures became shrunken and almost unrecognizable in fixed specimens. In living worms, it was also possible to observe at a glance the unusual arrangement of the longitudinal excretory canals on the aporal side. Thus it was determined beyond question that the large canal on the aporal side turns ventrally in the scolex, instead of dorsally as on the poral side, and the small canal, ordinarily termed dorsal because of its position, maintains throughout the strobila a position ventral to that of the large canal.

Hsü's diagnosis of the genus *Ophiovalipora* is emended as follows to include the additional species which should be assigned to it:

The Genus *Ophiovalipora* Hsü, 1935

With the characters of the subfamily Dilepidinae. Rostellum armed with a double crown of hooks, 9 or 10 to each circlet. Genital pores unilateral; genital atrium deep. Genital ducts pass between the longitudinal excretory canals and dorsal to the nerve. Testes dorsal to the ovary, arranged in a semicircle extending from the cirrus on the poral side, posteriorly around the female complex to or slightly beyond the anterior margin of the ovary on the aporal side. Excretory canals with the usual disposition on the poral side, but with the ventral one displaced dorsally on the aporal side and lying dorsal to the smaller canal. Each proglottid with a small, oblique transverse canal connecting the large ducts. Uterus sacciform, lobed. Parasites of birds and ophidians (?). Type species, *Ophiovalipora houdemeri* Hsü, 1935.

From the foregoing diagnosis and Hsü's excellent description of the type, it is apparent that the species described by Olsen (1937 a and b), as well as the one at hand, should be assigned to the genus *Ophiovalipora*. Their correct designations are accordingly:

Ophiovalipora lintoni (Olsen, 1937) comb. nov.
Syn. *Dendrouterina lintoni* Olsen, 1937b.
Dilepis unilateralis Linton, 1927, *nec* Rudolphi, 1819.
Ophiovalipora nycticoracis (Olsen, 1937) comb. nov.
Syn. *Dendrouterina nycticoracis* Olsen, 1937a.
Ophiovalipora minuta sp. nov.

Description of *Ophiovalipora minuta* sp. nov.
(Figures 1–10)

Specific diagnosis: (All measurements in millimeters.) With the characters of the genus. Small cestodes not exceeding 3.0 in length when well extended. Strobila with less than 40 proglottids, gravid segments not exceeding 0.42 in breadth. Scolex easily lost, 0.144–0.273 wide at the level of the suckers which measure from 0.08 to 0.105 long and 0.042 to 0.056 wide. Rostellum 0.044–0.064 wide, armed with a double crown of hooks, 10 in each row. Hooks of anterior row 0.035–0.04 long, posterior row 0.017–0.02 long. Genital pores within the anterior half of the proglottid. Cirrus sac often extends to the middle of the proglottid, 0.08–0.144 long and 0.028–0.035 wide. Basal part of the cirrus armed with minute spines 0.002 long. Testes usually 8 or 9 in number, tending to become diffuse and dissociate in mature proglottids in which as many as 11 masses have been counted; rarely as few as 5 observed. Vas deferens extremely coiled and surrounded by prominent cells of glandular appearance. Vagina ventral to cirrus;

vaginal sphincter well developed. Ovary bilobed, well developed, and apparently functional over an extent of not more than 5 proglottids. Excretory system typical of the genus; diameter of the large longitudinal canal 0.002. Transverse canal inconspicuous, visible only in sections and living material. Uterus visible only after eggs have developed in the gravid proglottids which never exceed 5 in number remaining attached to the strobila. Eggs variable in shape, from 0.041 to 0.052 in diameter; outer shell thin and delicate, well separated from the second covering which is composed of small spherical granules of uniform size. Within this is a mass of large and small refractile bodies which, under coverglass pressure, seem to form a distinct innermost layer enclosing the hexacanth larva. Embryos active when flattened; near the base of the hooks there often is a vacuole-like structure always containing a single nucleus-like inclusion. Embryonal hooks slender, 0.009 long.

Host: Butorides virescens L.
Locality: Tippecanoe County, Indiana, U. S. A.
Type specimen: Holotype no. 37139, Helminthological Collection, U. S. National Museum.

Ophiovalipora minuta is readily differentiated from all other members of the genus except *O. lintoni* which it resembles closely. This resemblance and the fact that both cestodes occur in the same host species raises the question as to whether the two are actually distinct. Indeed, the writer was unable to reach a decision in this matter until a large number of individuals had been studied and the material at hand had been compared with material of *O. lintoni,* including co-type 7866 from the Helminthological Collection of the U. S. National Museum and several additional specimens provided by R. Rausch. The descriptions given by Olsen (1937b) and Linton (1927) of *O. lintoni* indicate that the species may vary considerably in the length of the strobila and number of testes. Both authors were in error in their interpretation of the excretory system.

O. minuta is not only smaller than *O. lintoni,* but is also more uniform in size. The greater variation of *O. lintoni* in this respect may be due to the possibility that immature specimens were included in measurements. It is of interest to note that Linton recovered his smallest specimens of *O. lintoni* from heavily infected birds. This might well have been due to the recognized effect of crowding on the size attained by intestinal helminths. However, no appreciable size difference was noted between *O. minuta* from a lightly infected bird which yielded about 25 worms and cestodes recovered from a bird harboring over 200 individuals. The minimum length recorded for *O. lintoni* (degree of maturity not stated) is almost twice the maximum (2.6) ever observed for *O. minuta.* Furthermore, the maximum width observed for gravid proglottids of *O. minuta* did not exceed two-thirds that of *O. lintoni.* These size differences cannot be attributed to crowding, degree of contraction during fixation, or to a difference in host species. Nor can there be any doubt as to the maturity of the specimens at hand since there always were numerous detached fully gravid proglottids in the intestine, the number often reaching several hundred in heavily infected birds.

Other less obvious differences between *O. minuta* and *O. lintoni* were determined by careful study of whole mounts and sections. Although in individual proglottids, the number of testes may be the same in the two species, the maximum number in *O. minuta* is less than that of *O. lintoni.* The usual number of testes in the specimens of *O. lintoni,* observed in this study, was about ten. Although Olsen listed seven to eight testes, Linton observed ten to twelve. Both Linton and Olsen described the vagina as a heavy-walled, glandular structure. This is not the case in *O. minuta* which has a thin-walled vagina. Although the vaginal sphincter is about the same size in both species, it is relatively larger and has more distinct muscle

fibers and limiting membrane in *O. minuta.* The large rostellar hooks of *O. lintoni* are slightly shorter than those of *O. minuta* which have a range of 0.035 to 0.040. By comparing Figures 4 and 8, one can readily see that the hooks of these two species differ slightly.

Although the arrangement of the excretory system in the genus *Ophiovalipora* is unusual, it is not unknown in other genera. Because such genera are diverse in other respects, the peculiar arrangement of the excretory canals cannot be considered to have a taxonomic significance of greater than generic rank. Instead, it would seem to be a specialized condition arising independently in various groups. At the present time, *Culcitella* Fuhrmann, 1906, *Idiogenes* Krabbe, 1869, and *Dendrouterina* Fuhrmann, 1912, are known to possess this arrangement. In respect to the disposition of the reproductive organs, *Valipora* Linton, 1927, *Dilepis* Weinland, 1858, *Dendrouterina* Fuhrmann, 1912, and *Gryporhynchus* Nordmann, 1832, are very similar to the genus *Ophiovalipora.*

Although the genera *Dilepis, Dendrouterina,* and *Ophiovalipora* may be closely related, they are nevertheless distinct as shown in Figure 10. In *Dilepis* (a) the excretory canals have the usual arrangement and the genital ducts pass dorsal to them; in *Dendrouterina* (b) the canals are reverse in position on the aporal side and the genital ducts pass dorsal to the longitudinal canals on the poral side; in *Ophiovalipora* (c) the canals are reversed as in *Dendrouterina,* but the genital ducts pass between the excretory canals on the poral side. Hence there is no justification for Linton's allocation of *O. lintoni* to the genus *Dilepis* or for Rausch's implication that this, and the other species described by Olsen, be referred to that genus. Rausch appears to have been correct, however, in excluding them from the genus *Dendrouterina* on the basis of Baer's re-examination of Fuhrmann's material.

Key to the Species of *OPHIOVALIPORA*

1. Small hooks of rostellum less than 0.015 mm 2
   Small hooks greater than 0.015 mm 3
2. Nine hooks in each row, strobila 10 to 17 mm long ..... *O. nycticoracis* (Olsen, 1937).
   Ten hooks in each row, strobila 68 mm long ..... *O. houdemeri* Hsü, 1935.
3. Mature strobila with less than 40 proglottids and less than 3 mm long .... *O. minuta* sp. nov.
   Mature strobila with more than 40 proglottids and greater than 3 mm.
   O. lintoni (Olsen, 1937).

SUMMARY

1. *Ophiovalipora minuta* sp. nov. is described from the green heron, *Butorides virescens,* collected in Tippecanoe County, Indiana, U. S. A. *Dendrouterina nycticoracis* and *D. lintoni* are transferred to the genus *Ophiovalipora* and a key to the species of that genus is given.

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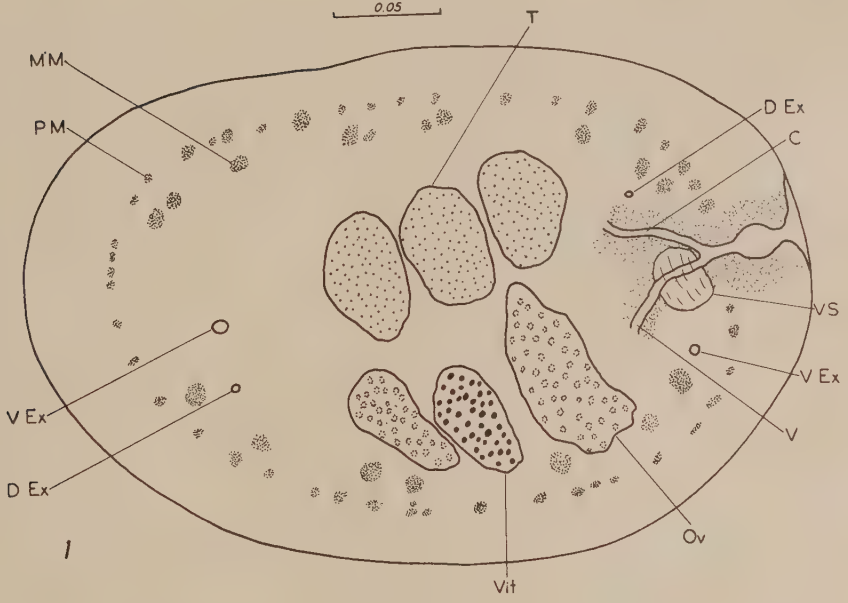
PLATE I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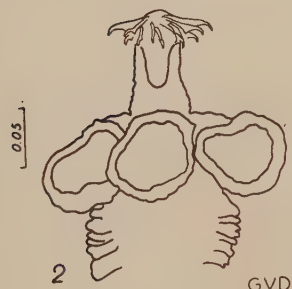
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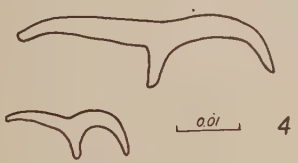
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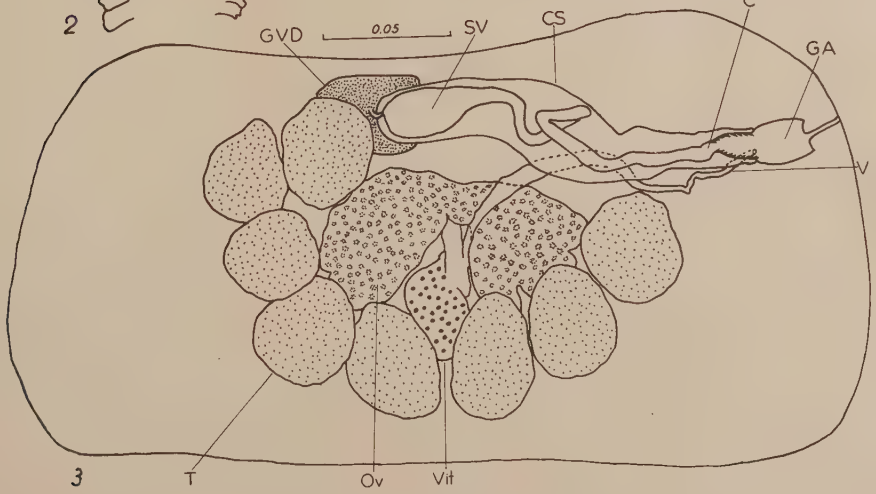
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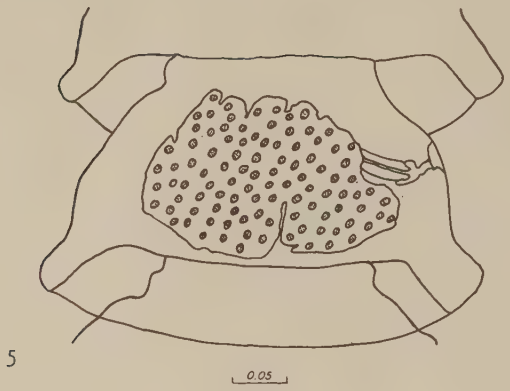

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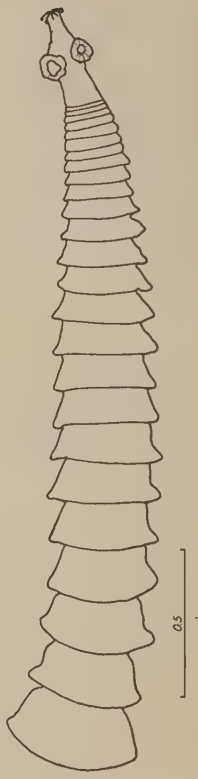

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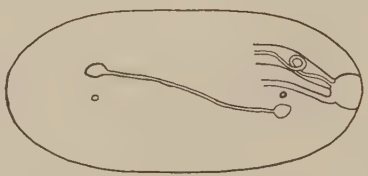

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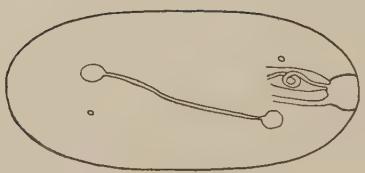

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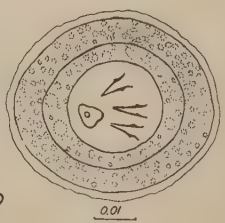

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LETTERING ON FIGURES

(All figures except Figs. 9 and 10 were drawn to the scales indicated with the aid of a microprojector.)

C Cirrus; CS Cirrus sac; D Ex Dorsal excretory canal; GA Genital atrium; GVD Glandular part of the vas deferens; MM Inner muscle bundles; Ov Ovary; PM Outer muscle bundles; SV Seminal vesicle; T Testes; V Vagina; V Ex Ventral excretory canals; Vit Vitellaria; VS Vaginal sphincter.

EXPLANATION OF PLATE I

FIG. 1. Transverse section of a mature proglottid of *Ophiovalipora minuta.*
FIG. 2. Scolex of *O. minuta.*
FIG. 3. Mature proglottid of *O. minuta.*
FIG. 4. Rostellar hooks of *O. minuta.*

EXPLANATION OF PLATE II

FIG. 5. Gravid proglottid of *O. minuta.*
FIG. 6. Transverse section of a gravid proglottid of *O. minuta.*
FIG. 7. Mature strobila of *O. minuta.*
FIG. 8. Rostellar hooks of *Ophiovalipora lintoni.*
FIG. 9. Mature egg of *O. minuta.*
FIG. 10. Diagram comparing the disposition of excretory canals and gonoducts in the genera (a) *Dilepis,* (b) *Dendrouterina,* (c) *Ophiovalipora.*

EUBRACHYLAELAPS DEBILIS, A NEW LAELAPTID MITE (ACARINA: LAELAPTIDAE) PARASITIC ON THE DEER MOUSE, *PEROMYSCUS MANICULATUS* (MAMMALIA: CRICETIDAE)

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The description of a new species of *Eubrachylaelaps* requires a clarification of the characters and limits of this genus, and also a consideration of *Cyclolaelaps,* a genus here synonymized with *Eubrachylaelaps.* These mites belong in the subfamily Laelaptinae; and are rather like *Laelaps* in their morphology and biology.

In this paper I refer only to females. Males of *Eubrachylaelaps* have never been described, and I have seen males of only two species. Ewing (1929) included in the generic diagnosis the statement that the genito-ventral plate bears a single pair of setae. This character alone is sufficient to separate *Eubrachylaelaps* from *Laelaps,* the latter genus having the genito-ventral plate with four pairs of setae. In the description of *Cyclolaelaps* the same character is mentioned (Ewing, 1933). Both descriptions mention the lack of a brush of setae on the chelicera; but in all species of Laelaptinae a row of fine setae subtends the digitus mobilis. There are no trenchant characters of generic rank by which to distinguish *Eubrachylaelaps* and *Cyclolaelaps.*

Eubrachylaelaps Ewing, 1929

Females

Body robust, almost circular. Sternal plate wider than long, bearing three pairs of setae, and with two pairs of slit-like pores. Endopodal plates each with a single seta, similar in size to the sternal setae. Parapodal plates confined to the region of coxae IV. Genito-ventral plate with one pair of setae. Metapodal plates small, void of setae. Anal plate with a pair of adanal setae and a single postanal seta. Scattered setae, sometimes in clearly defined rows, elsewhere on the venter; the more marginal setae are very slightly fringed. Dorsal plate in one piece, nearly circular, almost covering the dorsum, clothed with simple setae, with a pair of slit-like pores and numerous circular pores; of the latter there are typically two pairs, larger than the others, on the latero-caudal margin. Epistome in three sections, the most anterior being the most delicate; on the anterior margin there are no teeth, but a small median projection is conspicuous. Hypostomal teeth in six rows, three to seven teeth in each row. Each arm of the chelicerae bears two teeth in addition to the terminal tooth; the digitus fixus bears a pilus dentilis, the shape of which is typical for each species. A brush of fine setae surrounds the base of the digitus mobilis; a single seta projects from the base of the digitus fixus. Tritosternum two-branched, the branches separating well above the basal segment; the branches are finely serrate. Peritreme entirely ventral, not connected caudally with the parapodal plate.

KEY TO FEMALES OF THE NORTH AMERICAN SPECIES OF *Eubrachylaelaps*

1. Second sternal pores inclined less than 45 degrees away from the transverse axis of the sternal plate; ventral setae in obscure rows 2
   Second sternal pores inclined 45 or more degrees away from the transverse axis of the sternal plate, and parallel with the axis of the latero-posterior production of the sternal plate; ventral setae in well-defined posteriorly diverging rows 3
2. Sternal plate 4½ times as wide as long ..... *debilis,* new species.
   Sternal plate 2 times as wide as long ..... *crowei* Jameson.

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3. Genito-ventral plate expanded posteriorly, evenly rounded; postanal seta about twice the size of adanal setae ..... *circularis* (Ewing).
Genito-ventral plate somewhat narrowed posteriorly, not evenly rounded; postanal seta subequal to adanal setae ..... *hollisteri* (Ewing).

Eubrachylaelaps is closely related to *Laelaps*. In North America these two genera parasitize mice of the family CRICETIDAE, *Eubrachylaelaps* occurring on white-footed mice and their allies (CRICETINAE) and *Laelaps* on voles (MICROTINAE) (Table 1). *Laelaps echidninus* Berlese and *L. nuttalli* Hirst are parasites of murid rodents (the brown rat and the house mouse, respectively), and are aliens in North America. *Laelaps liomydis* Grant and *L. aplodontiae* Jellison do not belong in *Laelaps*.

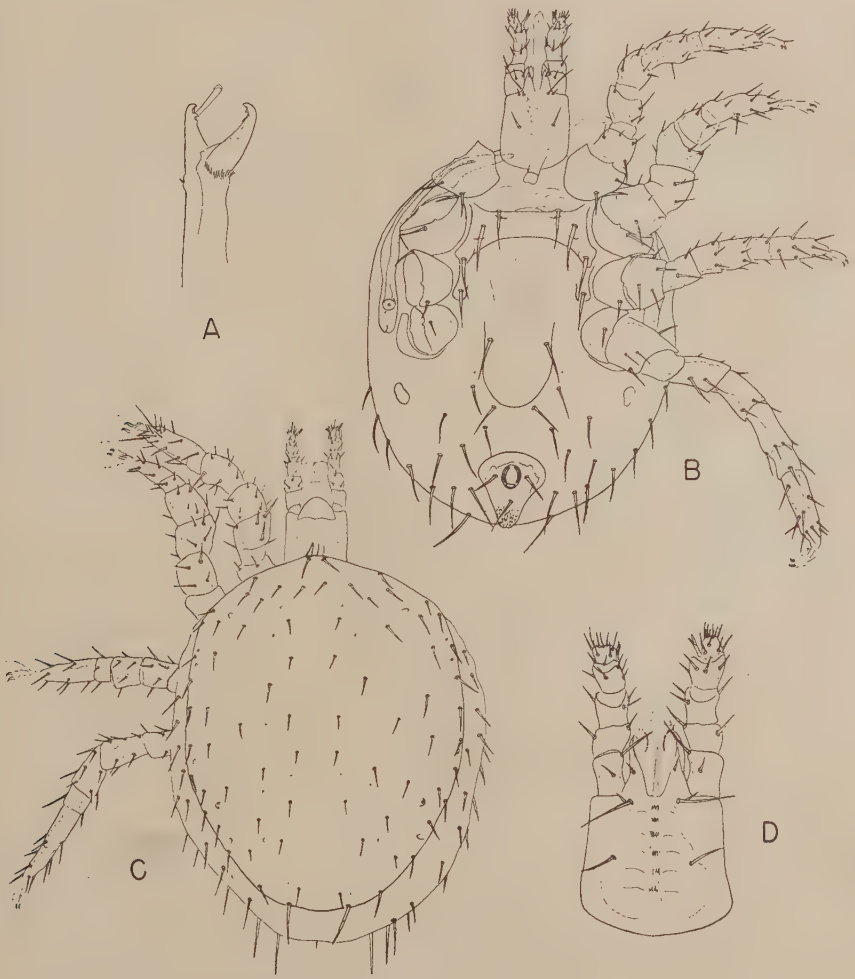

FIG. 1. *Eubrachylaelaps debilis*, new species. A, chelicera; B, venter; C, dorsum; D, gnathasoma.

TABLE 1.—*Host distribution of North American species of* Laelaps and Eubrachylaelaps

Parasite	Host
Laelaps kochi Oudemans	*Microtus* and *Pitymys*
L. alaskensis Grant	*Microtus* and *Clethrionomys*
L. multispinosus Banks	*Ondatra*
Eubrachylaelaps hollisteri (Ewing)	*Peromyscus californicus*
E. circularis (Ewing)	*P. truei, P. boylii, and P. hylocetes*
E. debilis, new species	*P. maniculatus*
E. crowei Jameson	*Onychomys leucogaster*

Eubrachylaelaps debilis, new species

Female

Venter (Fig. 1, B). Sternal plate about four and one-half times as wide as long, strongly concave caudally, the caudal margin reaching forward to the level of the second pair of sternal pores. Three pairs of sternal setae: first pair on slight projections of the anterior margin (in a few specimens actually in front of the sternal plate); second and third pairs in caudally diverging rows. Two pairs of slit-like sternal pores; in freshly molted specimens a third pair, near the third pair of sternal setae, is evident: first and second pairs of pores behind first and second pairs of setae, respectively; the pores subparallel to the transverse of the sternal plate. Endopodal plates well defined laterally, each bearing a single seta. Genito-ventral plate moderate for the genus, separated from the anal plate by a distance equal to two or more times the length of the anal slit; genito-ventral setae slightly longer than the endopodal setae. Anal plate roughly triangular, convex anteriorly, concave laterally; postanal seta only slightly larger than the adanal setae. Two or three pairs of small, amorphous metapodal plates, the largest of which are shown in the figure. Soft parts with setae in two pairs of poorly defined posteriorly diverging rows; two or three setae in the most anterior row, five in the second, and one or two additional setae on each side of the anal plate. The most caudal setae are slightly fringed. On each side of the genito-ventral plate is a small circular pore.

Peritreme extending from coxa IV to a point above the gnathasoma; there is a small pore immediately behind the stigma. Post-stigmatal part of peritreme not narrowed, but bluntly rounded, cleft at the apex.

Dorsum (Fig. 1, C): Dorsal plate circular, shoulders poorly defined. Dorsal plate with 39–40 pairs of setae of approximately equal size; only the last pair is enlarged. Behind the two anterior pairs of setae is a pair of slit-like pores; about ten pairs of circular pores. Soft parts with about ten pairs of setae.

Gnathasoma (Fig. 1, D): Hypostomal teeth in six rows, each row with three or four (rarely five) teeth. Lingula short and rounded. Chelicerae (Fig. 1, A) characteristic of the genus; pilus dentilus gradually broadened apically.

Type host: Peromyscus maniculatus (Wagner), deer mouse.

Type locality: Quincy, Plumas County, California.

Type: Holotype female; 23 February 1949; deposited with the U. S. National Museum.

Paratypes: Eight females with the same data as the types; twelve females from type host at type locality, 29 July 1949.

Additional specimens have been examined from Tuolumne County, California and from Clatsop and Washington Counties, Oregon.

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DESCRIPTION OF THE MALE OF *IXODES WOODI* BISHOPP (ACARINA: IXODIDAE)[1]

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United States Public Health Service

Among ticks received for identification in 1946 from Charles R. Joyce, U. S. Public Health Service Quarantine Station, Brownsville, Texas, was a single specimen of the previously unknown male of *Ixodes woodi* Bishopp, 1911. This specimen was found with a nymph of the same species in the den of a wood rat, *Neotoma* sp., December 7, 1945 and is described below. The material, all collected in the vicinity of Brownsville, also included four females of the same species, one from a wood rat den February 4, 1946, and three from a wood rat February 25, 1946. The original description of *woodi* was based on female specimens from *Neotoma micropus* Baird, Sabinal, Texas.

Ixodes woodi Bishopp
Male (neallotype)

Fig. 1

Body: Suboval, widest at about the middle. Length, tips of scapulae to posterior margin, 2.22[2]; width, 1.44. Color brown.

[2] All measurements are in millimeters.

Capitulum: Length, tips of palpi to posterior margin of basis, 0.39; greatest width of basis, 0.27. Lateral margins of basis rather abruptly narrowed behind the palpi, posterior margin salient and slightly concave, posterolateral corners rounded, cornua absent. Palpi short and broad, rounded dorsally; articles 2 and 3 about equal in length. Ventrally the surface of the basis is convex. Auriculae distinct as broad lateral saliences. Posterior margin rounded. Palpi flattened on their inner faces.

Hypostome: Notched apically. When the hypostome is examined before mounting the denticles appear as crenulations, but when mounted they appear as rather definite, short teeth in a 4/4 arrangement. Length about 0.164.

Scutum: About equally convex in the anterior and posterior areas, scapular areas a little elevated. Anterolateral areas faintly rugose. Scapulae short and rounded. Cervical grooves absent. Punctations about evenly distributed throughout, somewhat larger and deeper in the median lateral areas. Pseudoscutum not well defined. Hairs few, short, and scattered, more numerous on the marginal body fold.

Legs: Moderate in length and size. Coxa I with a distinct short, pointed, internal spur. Coxae II, III, and IV with salient corners in place of internal spurs. External spurs on all coxae small. All tarsi abruptly narrowed distally. Length of tarsus I, 0.42; metatarsus, 0.31. Length of tarsus IV, 0.42; metatarsus, 0.33.

Ventral plates: Median plate a little longer than the anal plate. Extreme length of the adanals about equal to that of median. Anal plate wider posteriorly, adanals wider anteriorly. All plates with a few inconspicuous punctations and hairs.

Genital aperture: Situated at the level of the intervals between coxae II and III.

Spiracular plate: Suboval, with the longer axis transverse. Greatest dimension, 0.21.

Deposited in the Rocky Mountain Laboratory, accession number 22078.

On the basis of the arrangement of the denticles in definite lineal files as observed in the mounted hypostome, the I. *woodi* male would key to I. *rugosus* Bishopp in Cooley and Kohls (1945) monograph. It is readily distinguished from

[1] Contribution from the Rocky Mountain Laboratory (Hamilton, Montana) of the National Institutes of Health.

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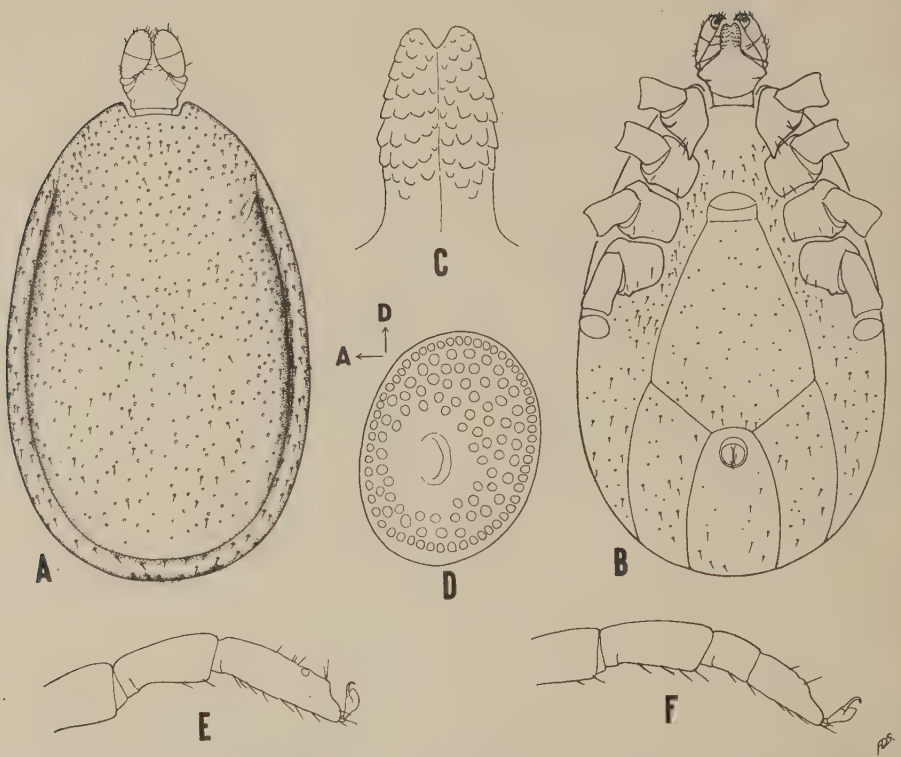

FIG. 1. *Ixodes woodi* Bishopp. Male. A. Capitulum and scutum, dorsum. B. Capitulum, coxae and ventral plates, venter. C. Hypostome. D. Spiracular plate (A = anterior, D = dorsal). E. Metatarsus and tarsus, leg I. F. Metatarsus and tarsus, leg IV.

that species, however, by its smaller size, the notched hypostome with the denticles arranged 4/4 rather than 3/3, and the absence of cervical grooves. Furthermore, all known collections of I. *woodi* are from Texas while those of I. *rugosus* are from the coastal region of Washington, Oregon, and California.

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DEVELOPMENT OF GERM CELLS IN THE ADULT STAGE OF THE DIGENETIC TREMATODE, *GORGODERINA ATTENUATA* STAFFORD, 1902.

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New York University, University College of Arts and Science

Information on the development of germ cells is available in the literature for only a few of the digenetic trematodes. Early studies centered around the question of whether or not the intercalary stages as well as the adult were involved in the production of gametes. Most workers today accept the adult trematode as the only form capable of forming gametes. Recent studies by White (1940), Jones (1945), and by Britt (1947) were concerned with gathering cytological evidence for an evolutionary mechanism and taxonomic relationships in the Platyhelminthes.

The present study describes the number and activity of the chromosomes in gametogenesis and during fertilization and early cleavage stages of the digenetic trematode, *Gorgoderina attenuata,* as well as observations pertaining to the nutritive processes in the egg and to the formation of its shell. *G. attenuata* belongs in the family Gorgoderidae and its life cycle was described by Rankin (1939). Twenty specimens from the bladders of *Rana pipiens* were fixed in Flemming's strong, aqueous corrosive sublimate-acetic, corrosive sublimate-alcohol mixture and Carnoy's chloroform-acetic-alcohol. Sections were cut from 5 to 8 μ and most were stained in Heidenhain's iron hematoxylin and counterstained with light green. Those worms fixed in the corrosive sublimate-alcohol mixture and in Carnoy's fluid were treated with Rafalko's (1946) modification of the Feulgen method. The basic fuchsin used was certified for Feulgen's technique, and sulphur dioxide was generated directly into the stain. The solution must be completely colorless. Three to ten minutes at 60 degrees C. was found to be the best time for hydrolysis. Washing was accomplished in a solution in which the sulphur dioxide had been previously generated directly into the water. Liang's (1947) alternative method, using sulphurous acid in making up the stain, was tried and gave good results. All drawings were made from sections with the aid of a camera lucida.

OBSERVATIONS

The worms were all sexually mature, the criterion being the presence of embryos in the uterus. They ranged from 1.75 to 3.5 mm. in length after fixation. The ovary, somewhat degenerate in one of the specimens, is almost spherical. The testes, absent in three specimens, are enveloped in a thin tunica of connective tissue and are somewhat elongate. The chromosomes in the spermatogonia, secondary spermatocytes, and secondary oöcytes are very short. In the spermatogonial late prophase two pairs of chromosomes measured about 3.0 microns, two pairs about 2.2 microns and three smaller pairs about 1.5 microns in length. When observed in the early cleavage stages, there is one J-shaped pair about 4.6 microns, one straight pair about 3.8 microns, one straight pair about 2.4 microns and four smaller pairs about 1.9 microns in length. The diploid chromosome number observed in spermatogonia

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and in early cleavage divisions is fourteen. The haploid number of seven was observed in primary and secondary spermatocytes and in primary oöcytes.

Spermatogonia. The primordial germ cells (Fig. 1) are irregularly clumped in closely packed groups at the periphery of the testis and may invade the interior for a short distance. Individual cell boundaries are not always clear. Spermatogonia, somewhat larger, appear among the primordial germ cells. A single nucleolus is usually present but it is difficult to establish any criteria that further distinguish spermatogonia from their antecedents. They vary from 3.5 to 5.3 μ in diameter. Primary, secondary and tertiary spermatogonia are irregularly distributed throughout the peripheral area of the testis. Occasional division stages such as the prophase in figure 2 were observed.

Spermatocytes. After the last gonial telophase, the spermatogonium enlarges and becomes the primary spermatocyte. The staining capacity of the nucleus increases and the chromatin begins to form strands, initiating the leptonema stage of the extended prophase period. The threads lengthen, become slender and are distributed throughout the nucleus (Fig. 4). They then arrange themselves into loops, with open ends oriented toward one pole of the nucleus in the characteristic "bouquet stage". A definite number of loops (Fig. 5) could not be counted nor could observations be made as to whether the thread is single or double at this stage. Synapsis (zygotene) was not observed. The chromosomes now undergo a marked shortening and are irregularly shaped (Fig. 7). Doubleness of the thickened thread at the diplonema was observed in only one case (Fig. 6) where the shortened threads had separated in the center. The chromosomes become progressively shorter and pass into diakinesis, toward the end of which the smooth contoured tetrads emerge (Fig. 8). The cells range from 5.3 to 8.3 μ in diameter at the early part of the extended prophase and from 8.3 to 11.3 μ in diameter when they emerge with tetrads as primary spermatocytes. They are spherical to slightly ovoid and are arranged radially. The group consists of eight cells, indicating that following division of the primordial germ cells the resultant spermatogonia have each divided twice. Crosses and ring-shaped tetrads are apparent, suggesting the presence of chiasmata, but the shortness of the chromosomes interferes with attempts to obtain more information. Seven tetrads are present (Fig. 7) and pseudoreduction has occurred. No evidence is available as to when true reduction takes place since the maternal and paternal chromosomes are not heteromorphic.

The groups of primary spermatocytes tend to lie nearer the center of the testis than the spermatogonia. The darkly staining tetrads align themselves on the spindle for the metaphase (Fig. 9). Spindle fibers and centrioles can be seen during this first maturation division. The tetrads divide in each of the eight cells which then pass through the anaphase and telophase stages producing a cluster of sixteen secondary spermatocytes, which are somewhat smaller than the primary spermatocytes. Cell delineations at the center of the cluster are indefinite. Each nucleus rounds up and undergoes the prophase activity resembling that of an ordinary mitosis prior to the second maturation division. The dyads in the secondary spermatocytes stain deeply and are ovoid (Figs. 10, 11, 13). They align themselves on the spindle and the chromatids pass to the poles (Fig. 10). Spindle fibers are evident but no centrioles could be seen. The resulting telophasic chromosome groups are small, compact and stain deeply (Fig. 12). The monoploid number of

chromosomes, seven, passes to each pole. This second meiotic division produces 32 spermatids which are arranged in a typical rosette-like pattern (Fig. 14).

Spermatids and spermiogenesis. Spermatids are smaller than secondary spermatocytes and are usually found in the central region of the testis. The resting spermatid nuclei appear granular and stain lightly. The region of the nucleus closest to the periphery of the cell becomes differentiated by staining more deeply and assuming the form of a crescent (Fig. 14). As the crescentic region elongates, the definitive nuclear outline disappears (Fig. 15). The intensely chromophilic condition subsides and the cell membranes slowly break down until an irregular mass of continuous cytoplasm appears with spermatozoa developing in the approximate regions where nuclei had been (Fig. 16). The residual cytoplasmic mass degenerates and the spermatozoa eventually pass into the vas deferens. They appear shorter and thicker when just released from the clusters in the testis than they do in the vas deferens.

Oögonia and oöcytes in the ovary. Primordial germ cells appear at the periphery of the ovary and oögonia are interspersed with them. They are closely packed together and in most areas only their nuclei are seen. No cell multiplication was observed in these early germ cells. The smaller nuclei in the peripheral region are in a resting state. Other nuclei, about 5 μ and larger, show nuclear activity (Fig. 18). The chromatin strands are thick, apparently continuous and stain deeply. As the nucleus further enlarges to a diameter of 12 μ, the threads thin out, become less chromophilic (Fig. 19), and vary in thickness presenting an irregular appearance. Finally the chromatin in the oögonium becomes diffuse and the cell, now a primary oöcyte, contains a large resting nucleus in the post-synaptic state. A nucleolus is present in the oögonial and pre-synaptic nuclei. It disappears during the chromatin activity and reappears in the post-synaptic stage. Occasionally two are present. The primary oöcytes with nuclei, which attain a size of 13.5 μ in diameter, are centrally located and make up the bulk of the ovary (Fig. 21). They are closely packed and irregular in shape due to pressure.

During the development of the oöcyte, dark hematoxylin-staining bodies appear in the cytoplasm. In the earlier stages they appear as two or three small contiguous droplets in the cells located near the periphery of the ovary. As the oöcyte enlarges these droplets appear to have coalesced, resulting in a large dark homogeneous mass in the cytoplasm at the periphery of the nucleus (Figs. 21, 22). The appearance of cellular components in the ovary after treatment with Feulgen technique is somewhat different. The active chromatin in the small peripheral cells stains deeply, while the primary post-synaptic nuclei contain diffuse, lightly staining Feulgen-positive granules. The nucleolus appears as a colorless region surrounded by these lightly staining granules and the extranuclear body which stained deeply with hematoxylin is a colorless mass.

An ovarian cavity develops into which the oöcytes pass, retaining their polygonal shape for a time. The entire cell is more chromophilic as it enters the oviduct and measures about 15.0 to 16.5 μ in diameter. According to Cort (1912), the oviduct widens into a fertilization space and from there it proceeds to the oötype region.

Fertilization and oöcyte nutrition. When the primary oöcyte proceeds into the fertilization space,it is surrounded by masses of spermatozoa. A single sperm enters the cell and remains completely inactive until the extended prophase begins (Fig.

43). The cell passes to the oötype region where through a short median duct, vitelline cells are released in groups from the vitellaria into the oviduct (Fig. 42). The nuclei of the vitelline cells contain many basophilic granules and the cytoplasm contains globules of an acidophilic substance. The nuclei react positively with Feulgen's technique while the cytoplasm and globules react negatively. A group of the vitelline cells adheres to one, occasionally to both ends of the oöcyte, and soon thereafter a shell membrane appears enclosing the oöcyte and the vitelline cells. At about the same time that the shell appears, the globules in the cytoplasm of the vitelline cells disappear and the remaining cytoplasm degenerates. Groups of vitelline nuclei and degenerated cells have been observed in portions of the uterus. The shell membrane reacts negatively to Feulgen's method. The egg at this stage measures 26.5 μ long and 15 μ wide. Although the median vitelline duct runs through the region of Mehlis' gland, there appeared no evidence that this gland has anything to do with the shell-forming process.

The hematoxylin staining mass in the cytoplasm of the oöcyte becomes less chromophilic and gradually disintegrates when the nucleus is in its extended prophase (Fig. 43). The chromatin granules in the nucleus stain deeply and once again form a strand. In Feulgen-treated material this strand was observed in a coiled condition and in loops (Fig. 25) suggesting a bouquet stage. The thread then breaks up into a number of shortened strands (Fig. 27). In what was interpreted as a diplotene stage (Fig. 43), a strand had opened at points along its length, showing evidence of having undergone synapsis. The chromosomes continue to shorten and assume the irregular shapes of diakinesis (Figs. 28, 32) and finally, smooth-contoured, deeply-staining, variformed tetrads appear (Fig. 33). The tetrads align themselves on the metaphase spindle and in the ensuing division a secondary oöcyte and first polar body result. The polocyte stains deeply and no positive observation could be made as to whether or not it contains any cytoplasm. It eventually becomes lost among the vitelline nuclei within the shell membrane. Small, ovate dyads (Fig. 37) appear in the secondary oöcyte, line up on the metaphase spindle and divide producing the egg and second polocyte (Figs. 36, 38). No evidence could be gathered as to which of the maturation divisions is reductional, but with the formation of the seven tetrads pseudoreduction has occurred.

Early cleavage. As the oöcyte nucleus begins its extended prophase, the resting sperm shortens and becomes almost spherical. After the first maturation division, the sperm enlarges again, its structure loosens and a network of strands appears (Fig. 38). Later it becomes vesicular and the chromatin becomes diffuse and scattered through the nuclear framework as the gamete passes into the male pronucleus stage. The female pronucleus enlarges and shows a loosening of the telophasic chromosomal clump. Both pronuclei remain in a resting state for a time (Fig. 39), after which they increase in staining capacity, chromatin granules increase in size and the nuclear membranes break down (Fig. 40). Seven chromosomes appear in each of the nuclei (Fig. 41). The first cleavage division was not observed, but in all 2-cell stages seen, one cell was larger than the other. In the second cleavage division only the larger of these cells divides, producing a 3-cell stage. Figure 44 illustrates a stage prior to the third cleavage division. The chromosomes figured here are representative of those in somatic tissues.

DISCUSSION

Gametogenesis in *Gorgoderina attenuata* is essentially similar to that described in other digenetic trematodes. Variations exist which may be attributed to species differences. Jones (1945) in a cytological study of cestodes using Feulgen's method, hydrolyzed his material at 40 degrees C. from 60 to 80 minutes and stated that the evidence of specificity for an optimum period of hydrolysis, indicated that an individual optimum might be attributed to each species. This might also apply to the different tissues within an organism. Britt (1947), using the Feulgen method on trematodes, hydrolyzed for 25 minutes but did not state the temperature. He sectioned his material at 12 μ in the belief that thick sections offer a better view of the complex being studied. However, while favoring the interpretation of long chromosomes, thick sections interfere with observation of finer cytological detail. Britt (1947) gave 14 as the diploid number of chromosomes for *G. attenuata,* the same as reported by Willey and Koulish (1947). Pennypacker (1936) observed that most of the chromosomes in the germinal tissues of *Pneumonoeces medioplexus* look alike, being ovoid or spherical. Rees (1939) in studies on gametogenesis in *Parorchis acanthus* also observed this, and stated that the chromosomes regain their true form in somatic tissues. The shapes of the chromosomes of *Gorgoderina attenuata* in the present study vary in the same manner.

In another species of the family Gorgoderidae, *Probolitrema californiense* Stunkard, Markell (1943) observed an irregular arrangement of the primary, secondary and tertiary spermatogonia in the testes, and their similarity to primordial germ cells, as here described for *Gorgoderina attenuata.* This spermatogonial behavior may be characteristic of the Gorgoderidae. The spermatogonia studied in other families of trematodes are grouped in twos, fours and eights as a result of three definite spermatogonial divisions.

Attempts to identify the stage during meiosis at which true reduction occurs, have led some authors to draw conclusions for which there is inadequate evidence. Chen (1937) reported that the first maturational division is reductional. Rees (1939) stated, "In spermatogenesis and oögenesis of the adult, the first meiotic division is the reduction division." Anderson (1935), describing the first meiotic division of the primary spermatocyte of *Proterometra macrostoma,* wrote, "With the beginning of the anaphase the separation of the synaptic mates is evident, and as the individual chromosomes proceed toward the poles, a longitudinal split in each becomes apparent (Fig. 35). This split is indicative of the division which occurs in the final (equational) division. Thus the tetrad nature of the bivalents in the metaphase is not truly apparent until the separation into the univalents of the anaphase." The question arises as to the evidence leading to the conclusion that the tetrad has divided along the plane between the synaptic mates (reduction) rather than in the plane of the longitudinal split (equation division). It has not been possible to distinguish maternal from paternal chromosomes in the monoecious Digenea, in which group heteromorphic chromosome pairs are as yet unknown. Therefore there is no evidence to indicate whether a tetrad is dividing reductionally or equationally. In his description of division of the primary oöcyte, Anderson (1935) stated, "There is little question, however, that this is the heterotypic or reductional division." No evidence is offered in support of this interpretation. It should be pointed out here that the term "heterotypic" as used in modern cyto-

logical literature means only the first meiotic division and is in no sense synonymous with "reductional division." Cable (1931) in studies on gametogenesis in *Cryptocotyle lingua,* and Willey and Godman (1941) in a description of spermatogenesis in *Zygocotyle lunata* suggested that reduction occurs during the first maturation division. Cable (1931) stated, "It is of general occurrence that the first maturational division is reductional and it is believed, for reasons already given, that *Cryptocotyle lingua* is no exception to this rule." Carothers (1926) in an analysis of maturation in its relation to reduction stated, "Thus the initial reduction in number of chromosomes occurs through the union (synapsis) of homologous or like chromosomes, one contributed by the egg, and one by the sperm, and not as a result of cell division. This process is known as pseudo- or false reduction, since the actual distribution to different cells is accomplished by the two following divisions. . . . The two maturation divisions are essentially a unit process and segregation may occur in either." In the light of this interpretation and in the absence of evidence showing the existence of heteromorphic homologues in the Digenea, statements identifying a meiotic division as reductional or equational are little more than conjecture in this group.

Anderson (1935) observed certain irregularities during gametogenesis in *Proterometra macrostoma.* He reported considerable numbers of pyknotic nuclei which disappear in later stages, and suggested that they are degenerating oöcytes which are later absorbed by the normal cells thus contributing to their nutrition. He stated, "Nuclear degeneration or pyknosis is frequently observed in cells in the testes and occasionally in the ovary. . . . The pyknotic cells are characterized by an extreme contraction of the entire nuclear content. . . . These may be distinguished from an extreme syndesis in synapsis by the fact that there are no signs of polarization, and the syndetic figure will always appear at one side of the nuclear area rather than in the center." In this quotation a confusion of terms obscures the meaning. The term 'syndesis' is synonymous with synapsis, and the term synizesis, meaning the contraction figure, should be subtituted for 'syndesis' in Anderson's paper. Groups of cells similar to the "degenerating oöcytes" of Anderson were observed in the testis of *Gorgoderina attenuata* (Fig. 3) but their significance is unknown. Markell (1943) suggested that the "residual cytoplasmic masses" and the "degenerating oöcytes" of Anderson are identical. Observations made on sections of *G. attenuata* in the present work lend no support to this view, since in this species, residual cytoplasmic masses degenerating after sperm have escaped are entirely distinct from the occasional oöcyte-shaped cells appearing in the testis.

The stages of oögenesis in trematodes have not been observed as fully as those of spermatogenesis. Cable (1931) reported that mitosis is rare in the ovary of *Cryptocotyle lingua.* No oögonial divisions were observed in the ovary of *G. attenuata.* Cells with nuclei in the post-synaptic diffuse stage are primary oöcytes resulting from growth and nuclear activity of oögonia. The hematoxylin staining mass found in the cytoplasm of the developing oöcyte is probably reserve food material. Similar bodies have been observed in *Probolitrema californiense* and in *Gorgoderina sp.* by Markell (1943). Woolcock (1935), studying sections of *Probolitrema antarcticus,* observed granules believed by her to be yolk granules in the cytoplasm of oöcytes. Pennypacker (1940) observed cytoplasmic granules in developing oöcytes of *Pneumonoeces similiplexus* and in *P. medioplexus* and dis-

cussed their possible relationship to the nucleus. If these masses contain reserve food material it is difficult to understand why they increase in size along with the growing oöcyte, unless they store up food material to be used later when the oöcyte leaves the ovary. Markell (1943) stated, "From the size of these inclusions when they first appear, and their gradual growth, it may be assumed that they are secreted in the cytoplasm of the growing oöcytes, to form a reserve source of nourishment which is resorbed in later stages." No degenerating cells or nuclei which might act as food for the oöcyte were observed in the ovary of *Gorgoderina attenuata*. Furthermore, these reserve food masses in the oöcytes stain negatively when treated with the Feulgen method, providing evidence against the belief that they are engulfed yolk nuclei as reported in earlier studies (Goldschmidt 1905), and against the belief of Anderson (1935) and others that they are the pyknotic nuclei of degenerating oöcytes absorbed by normal oöcytes.

Observations on the vitelline cells of *Gorgoderina attenuata* show that globules of various sizes, taking the acid stain, rather than granules are precursors of the egg-shell. Rees (1939) described this substance as globular in *Parorchis acanthus*, while Markell (1943) observed formation of the egg-shell membrane from granules in the vitelline cells of *Probolitrema californiense*. In *G. attenuata*, the shell forms soon after the application of the vitelline cells to the oöcyte, probably by the breakdown of the cells and the release of the globules. According to Markell (1943), "Apparently normal shells are formed only in the Mehlis' gland region, and if ova and vitelline cells pass through this part of the oviduct too rapidly, or fail to arrive there simultaneously, a shell will not be formed. This may be due to some action of the secretion of Mehlis' gland, or merely to mechanical factors." That Mehlis' gland plays some role in formation of the shell seems indicated by the fact that in some trematodes which lack the gland the egg shells are thin and membranous. Stunkard (1943) stated that in *Zoögonoides laevis*, "There is no "shell gland" and the miracidia develop in thin-walled, membranous sacs. . . . The egg membrane is flexible and the shape varies with pressure."

Stephenson (1947) after histochemical analyses of the mature, brown egg-shell and the earlier colorless shells and of vitelline cell granules of *Fasciola hepatica*, confirmed Vialli's (1933) findings that the vitelline granules contain an ortho-dihydroxyphenol. Reasoning from the report of Pryor (1940), that sclerotin is a quinone tanned protein and that quinone is derived from oxidation of an ortho-dihydroxyphenol, Stephenson concluded that the egg shell of *F. hepatica* is similar to sclerotin in all properties thus far investigated. He thought that Mehlis' gland might secrete a protein constituent of the egg-shell, but using histochemical methods, he obtained negative results for the presence of such a substance and concluded that globules or granules contained in vitelline cells probably furnish all the protein polyphenols required for sclerotin formation. He stated, "As the egg traverses the uterus, it shrinks, becomes brown, hard, brittle, and relatively impermeable. This is due to the oxidation of the polyphenol of the newly formed egg shell to give a quinone, which links to the protein to give a quinone tanned leather."

In *Gorgoderina attenuata* the sperm enters the oöcyte before the application of either vitelline cells or shell and before the oöcyte has reached the level of the median vitelline duct. Anderson (1935) stated that in *Proterometra macrostoma* the sperm does not enter until the shell is nearly completed. Rees (1939) observed that in all

cases the yolk cells became applied to the oöcyte of *Parorchis acanthus* prior to the entrance of the sperm. Chen (1937) found that the sperm entered prior to the application of both yolk and shell in *Paragonimus kellicotti.*

Some variation exists in the history of the pronuclei prior to the first cleavage in trematodes. Von Kemnitz (1913) asserted that in *Brachycoelium* the pronuclei fused before the first cleavage. He based his opinion on the presence in the fusion nucleus of two nucleoli. Cable (1931) observed the presence of two pronuclei of equal size, while Markell (1943) stated that of the two the male pronucleus is the smaller. Rees (1939) described two pronuclei which form simultaneously and remain in a resting stage for a while. In all of these studies as well as in that of Chen (1937), the pronuclei after fusion pass into a resting stage before entering the prophase of the first cleavage mitosis. Jones, Mounts and Wolcott (1945), tracing the history of the pronuclei in *Macravestibulum kepneri,* asserted that the pronuclei, each with a single nucleolus, are in prophase at the time of their union and that the chromosomes pass directly to the metaphase of the first cleavage division without undergoing a resting stage. In *G. attenuata* male and female pronuclei were observed in both resting and prophase stages prior to nuclear fusion. The presence of more than one nucleolus in these cells is a poor criterion for fusion of pronuclei, because many cells in earlier stages have been observed to contain more than one nucleolus.

The first cleavage has been described as unequal in *Polystomum integerrimum* by Goldschmidt (1902), in *Fasciolopsis buski* by Ishii (1934), in *Paragonimus kellicotti* by Chen (1937), in *Parorchis acanthus* by Rees (1939) and in *Zygocotyle lunata* by Willey and Godman (1941), resulting in two blastomeres of unequal size. Observations on *G. attenuata* are in complete agreement showing two unequal cells. The second cleavage involves only the larger cell and results in a three-cell stage. Although no mention is made of it in their text, Jones, Mounts and Wolcott (1945) show a figure of a two-celled embryo in which the cells are of equal size. This would seem to be an important difference in the light of the interpretation of Chen (1937) and Ishii (1934) that the smaller cell is a "propagatory" or "stem" cell, representing the earliest separation of germinal from somatic cells in the trematode embryo.

SUMMARY

Development of male and female gametes has been traced cytologically from primordial germ cells to maturity in *Gorgoderina attenuata.* The diploid chromosome number is 14, and 7 chromosomes appear in each of the mature gametes. Cytoplasmic constituents as well as nuclear structures have been studied with regard to their origin and fate. The Feulgen technique has been of value in the determination of certain cellular components. Fertilization and early cleavage are described. The relation between cytoplasmic structures of oöcytes and vitelline cells and the nutrition and shell formation of the egg have been studied. The results have been correlated with descriptions of these processes in other species.

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Explanation of Figures

Plate I. *Gorgoderina attenuata,* Figs. 1–17.

Fig. 1. Primordial germ cells and spermatogonia in testis.
Fig. 2. Spermatogonium showing chromosomes in late prophase.
Fig. 3. Group of degenerating cells in testis.
Fig. 4. Young spermatocyte showing early leptotene threads.
Fig. 5. Bouquet stage.
Fig. 6. Diakinesis.
Figs. 7, 8. Tetrads in primary spermatocyte.
Fig. 9. Metaphase of first maturation division.
Fig. 10. Metaphase and telophase of second maturation division.
Fig. 11. Late prophase in secondary spermatocytes.
Fig. 12. Telophase in secondary spermatocytes.
Fig. 13. Late prophase and metaphase in secondary spermatocytes.
Figs. 14–17. Development of spermatids into spermatozoa.

Plate II. *Gorgoderina attenuata,* Figs. 18–33.

Fig. 18. Primordial germ cells and oögonia at periphery of ovary.
Fig. 19. Enlarged oögonia with chromonemata breaking up.
Fig. 20. Primary oöcytes containing post-synaptic nuclei with prominent nucleoli (nu).
Fig. 21. Primary oöcytes showing reserve food material (rf) in the cytoplasm.
Fig. 22. Primary oöcytes just prior to entrance into oviduct.
Fig. 23. Primary oöcytes in the oviduct.
Fig. 24. Primary oöcyte containing sperm.
Fig. 25. Primary oöcyte showing portion of thread in "bouquet". Feulgen stain.
Fig. 26. Chromonemata as coiled threads. Feulgen.
Fig. 27. Condensation into short strands. Feulgen.
Fig. 28. Late prophase prior to tetrad formation. Feulgen.
Fig. 29. Tetrads in first maturation division of oöcyte.
Fig. 30. Dyads in secondary oöcyte. Feulgen.
Fig. 31. Primary oöcyte containing sperm.
Fig. 32. Shortened and twisted chromonemata during prophase in primary oöcyte. Feulgen.
Fig. 33. Primary oöcyte showing seven tetrads.

Plate III. *Gorgoderina attenuata,* Figs. 34–44.

Fig. 34. Oöcyte in metaphase of first division. The sperm (sp) is shortened and condensed into a spherical mass.
Fig. 35. Anaphase of first maturation division. Shell membrane (sh) is forming and some vitelline cells are broken down.
Fig. 36. Metaphase of second maturation division.
Fig. 37. Secondary oöcyte showing dyads and sperm. Feulgen.
Fig. 38. Telophase of secondary oöcyte showing first polar body (pb 1) and enlarging sperm (sp).
Fig. 39. Egg with male and female pronuclei in resting stage.
Fig. 40. Prophases of male and female pronuclei.
Fig. 41. Haploid chromosome sets of male and female pronuclei, prior to metaphase of first cleavage.
Fig. 42. Vitelline cells showing globules (gl) of shell-forming substance.
Fig. 43. Primary oöcyte in diplotene. Sperm has entered and the reserve food mass (rf) is breaking down.
Fig. 44. Late prophase of third cleavage, showing chromosomes characteristic of somatic tissues in embryo and adult.

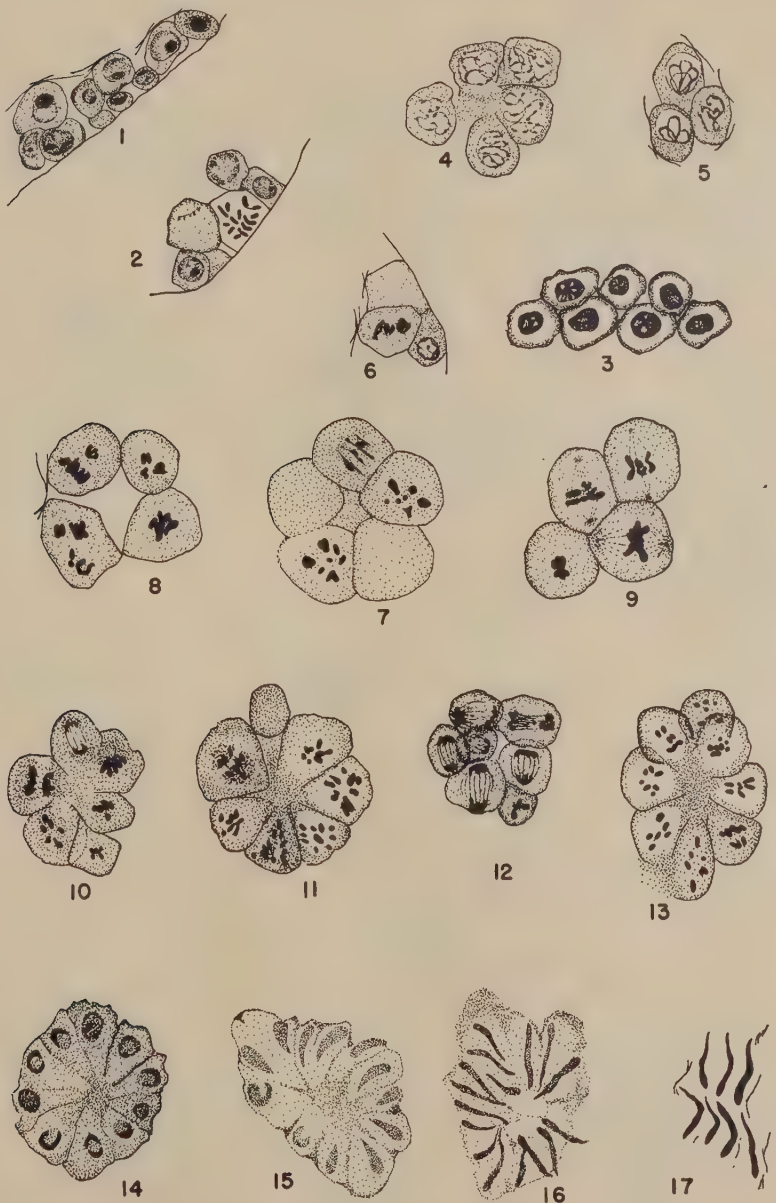

PLATE I

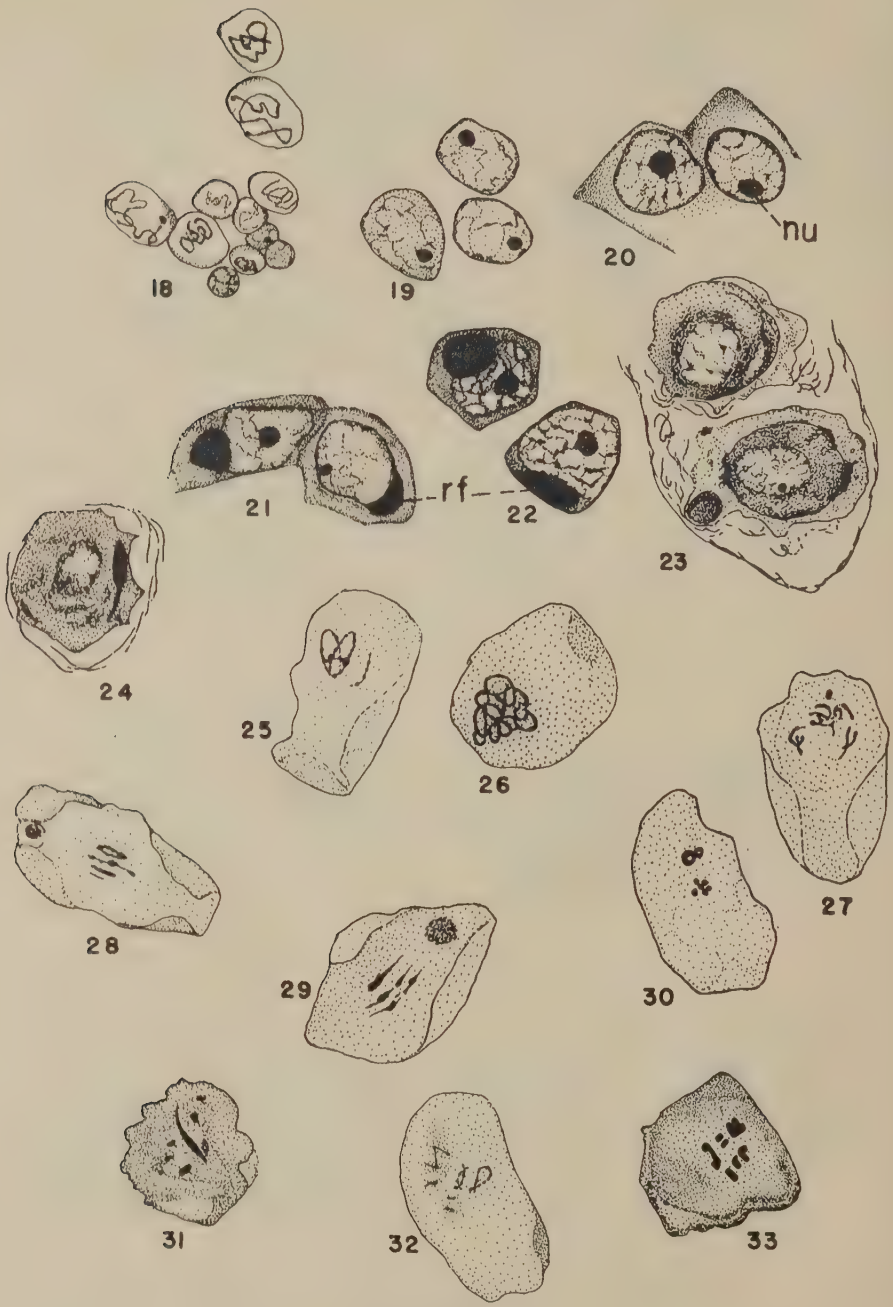

PLATE 2

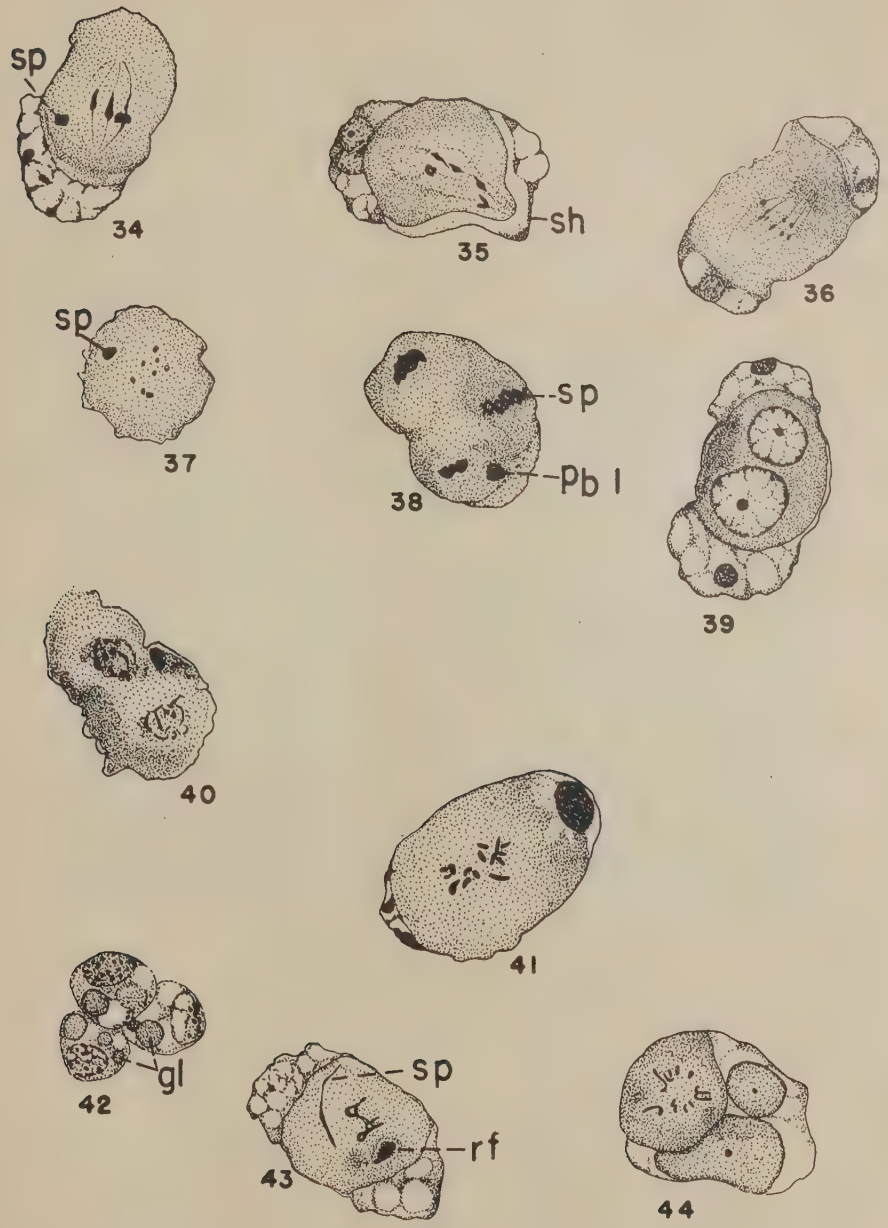

PLATE 3

A NEW SPECIES OF JAPANESE LARVAL MITE FROM A NEW FOCUS OF TSUTSUGAMUSHI DISEASE IN SOUTHEASTERN HONSHU, JAPAN*

TSUGUO KUWATA, TRYGVE O. BERGE AND CORNELIUS B. PHILIP

Incidental to epidemiological studies in the Fujino Susuno Maneuver Area near the base of Mount Fuji southwest of Tokyo, where a number of U. S. troops on field exercise contracted tsutsugamushi disease in October, 1948 (as reported in Ref. No. 1), samples of mites were recovered from indigenous small animal hosts for determination and attempted recovery of rickettsial strains. Collections were

Trombicula (*Leptotrombidium*) *fuji* n. sp.

Belonging to the "tsutsugamushi group" of chigger mites, this species is distinguished by its very sparsely punctate, unusually small scutum with the sensillary bases well behind the line of the postero-lateral setae, the latter situated in advance of the median line, the sensillae feathered on the outer two-thirds, barbed on the distal half of the shank, and a bare seta on palpal segment III which exceeds the tip of the palpal claw in length (Fig 1).

FIG. 1. *Trombicula fuji* n. sp. Diagrams of dorso-ventral aspects, of palpus and of scutum.

Size of fully engorged larva, 515×670μ. Color in life, pale reddish-yellow.
Capitulum: Cheliceral base longer than wide, about 18 sparse punctae (Fig. 2). Galeal

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made in late October and mid-November. Host species examined were the far eastern field mouse *Apodemus speciosus speciosus* Temminck and Schlegel, the field vole *Microtus montebelli montebelli* Milne-Edwards, and the northern mole shrew *Urotrichus talpoides hondonis* Thomas. The mite samples included *Trombicula scutellaris, T. palpalis, T. pallida, T. intermedia, Gahrliepia* sp., and the following undescribed species recovered from *Apodemus speciosus.*

In collections made on 3 and 4 June 1949, in the same (Gotemba) area, 48 additional *T. fuji* were recovered from *A. speciosus,* and on 4 June, 9 *T. fuji* from *Apodemus geisha* Thomas. In both cases, these were the predominant mite species encountered on the living animals. In addition, more than 250 mites from the same hosts were recovered for rearing experiments.

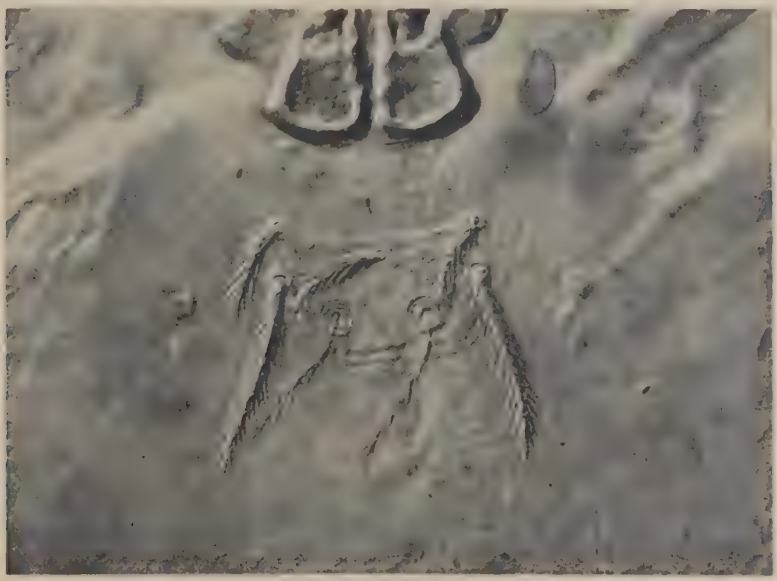

FIG. 2. *Trombicula fuji* n. sp. Photomicrograph of scutum, eyes and cheliceral bases.

setae feathered. Setae: on palpal segment I—feathered, II—bare, III—bare and very long, IV—dorsal feathered, lateral and ventral bare.

Scutum: Exceptionally small, suboblongate with rounded caudal corners, the anterior angles acutely rounded at the bases of the anterolateral setae. Punctations few, fine and scattered on the disc between and in advance of the sensillary bases. All setae feathered; AM and AL subequal in length, PL about twice as long, and latter a little longer than the sensillae.

Dorsum: Eyes 2/2, unusually small, the posterior pair vestigial, no ocular plate evident. Dorsal setal counts fairly constant: (type) 2, 8, 6, 6, 2, 6, 2 (counts on paratypes are 2, 8, 5–6, 5–6, 3–4, 3–4, 0–3; 30–32 total setae.) All setae are sparsely barbed.

Venter: Two pairs of sternal setae plus about 24 small preanal and 18 larger postanal, sparsely barbed setae.

Legs: Seven segments on all and the usual trifurcate claws. Coxal setae, 1–1–1, feathered; vestiture of other segments not unusual; no long, bare, whip-like setae on the hind legs.

Type data: Holotype and 9 paratypes on separate slides, from field mouse *Apodemus speciosus,* taken on lower, east slope of Mount Fuji, Fujino Susuno (Gotemba) focus, 30 October, 1948, Major Trygve O. Berge. Holotype in the U. S. National Museum, No. 1863; paratypes in collections of the Rocky Mountain Laboratory, Hamilton, Montana, British Museum, South Australian Museum, and authors.

COMMENTS

Segi and Takagi (1923) described a species of mite termed "B" collected from field voles in Shizuoka prefecture which bears some resemblance to *T. fuji* but differs in showing a wider scutum, distinct double eyes with ocular plate, and three nude setae on palpal segment IV.

Philip and Tamiya (1946) first mentioned the possible role of *A. speciosus* as a mite host in the dissemination of tsutsugamushi disease in Honshu. This species and related species of field mice have been found to be abundant in the Fuji endemic areas so far surveyed.

In the key to Japanese vole mites by Philip (1947), *T. fuji* would key out with *T. pallida*, from which it is at once distinguished by the smaller scutum, longer but less heavily branched and fewer dorsal setae, SB farther behind PL, longer seta on palpal segment III, etc.

Since the Standard Measurements computed by Womersley and Heaslip (1943) from published figures are smaller than observed for *T. scutellaris* and *T. intermedia*, actual data are provided in Table II, and the scuta illustrated in Figures 3 and 4.

The absence of the classical Japanese vector, *T. akamushi*, in the mite samples from these new foci in 2 different years is noteworthy, and appears doubtfully attributable to seasonal factors at times of survey.

TABLE I.—*Trombicula fuji, n. sp. Standard Measurements of Holotype and Extremes of Eight Paratypes in* μ.

	AW	*PW*	*SB*	*AP*	*SD*	*PSB*	*PL-SB*	*AM*	*AL*	*PL*	*S*	*HM*	*DSA*	*DSP*
Type	50.5	52	24	14	30.5	11	10.5	29.5	28.5	51	40.5	45	50	34
Extremes	44–50	48–55	21–24	13–14	30–37	10–15	10–11	26–30	23–27	45–48	33–40	40–47	47–51	33–43
Mean	49	51.38	23	13.4	33.98	12.2	10.2	27.5 (6)	25.5	46.8	35.9 (6)	43.5	49.6	35.6

TABLE II.—*Standard Measurements of 5* T. intermedia *and 5* T. scutellaris

		AW	*PW*	*AP*	*SB*	*PSB*	*AM*	*AL*	*PL*	*S*
T. intermedia	1	71.5	78.5	24.5	34	12.5	50.5	41.5	55	62
" "	2	69	79	23.5	33.5	12.5	49?	38?	54.5	59?
" "	3	68.5	77	23.5	33.5	13.5	..	36.5	53.5	67
" "	4	70	77	22	33.5	13.5	50	37	57	58
" "	5	67	77.5	24	34	13.5	..	43.5	54	..
T. scutellaris	1*	74	83.5	31	31.5	16.5	54.5	43	57	83.5
" "	2*	66.5	79	28	29.5	15.5	55.5	48	59.5	..
" "	3*	67	78	32	30	20	60.5	54	60.5	81.5
" "	4#	69.5	82.5	31.5	31	19.5	62	52.5	64	76.5
" "	5#	76	88	31	31	19	55	48	64	..

* Mt. Fuji area, 1948. # Yamagata area, 1945.

SUMMARY

Trombicula (*Leptotrombidium*) *fuji n.* sp. of larval field-mouse mite from a new endemic focus of tsutsugamushi disease on slopes of Mt. Fuji, Japan, is described. Holotype in U. S. National Museum No. 1803. Standard measurements of larvae of *T. intermedia* and *T. scutellaris* are also provided. All three species belong in the "tsutsugamushi group" of mites.

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(Photomicrographs by N. J. Kramis, Hamilton, Montana)

FIG. 3. *Trombicula scutellaris*, Nagayo et al. Photomicrograph of scutum.

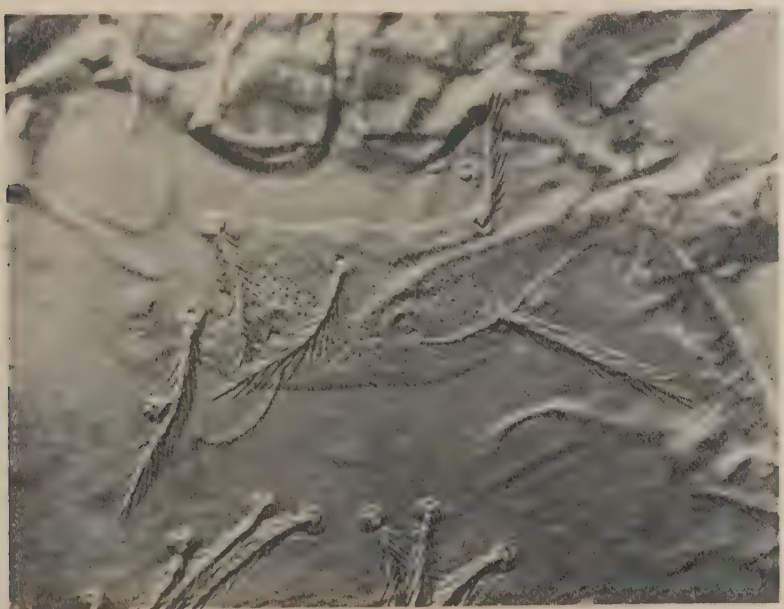

FIG. 4. *Trombicula intermedia*, Nagayo et al. Photomicrograph of scutum.

RESEARCH NOTES

NEW HOST AND DISTRIBUTION RECORDS FOR TWO TREMATODES FROM THE WESTERN GULL

During January and February, 1949, four Western gulls (*Larus occidentalis*) were killed at Newport, Oregon. Nine specimens of *Parorchis acanthus* were found in the rectums of two of the gulls. The gall bladders of three gulls yielded 56 trematodes (*Gymnophallus delicious*).

According to Dawes (The Trematoda, Cambridge Univ. Press, Cambridge, pp. 357–8, 1946), *Parorchis acanthus* has been found in the herring gull, common gull, and flamingo; he gave the distribution as from both sides of the Atlantic Ocean. Finding this trematode in the Western gull represents a new host and distribution record for *P. acanthus.*

The life cycle of *Parorchis avitus* (=*P. acanthus*) was worked out by Stunkard and Cable (Biol. Bull. 62: 328–338) at Woods Hole Marine Biological Laboratory in 1932. They stated that the cercariae were found in *Urosalpinx cinereus* and *Thais lapillus.* Snails were collected at Newport Feb. 26, 1949; the following species were examined: 368 *Thais emarginata,* 22 *T. caniculata,* and 46 *Tegula funebralis.* Rediae and cercariae, which agreed with the description given by Stunkard and Cable, were found in two specimens of *T. emarginata.* Examination of 116 *Thais emarginata* collected April 17, 1949, at Fogerty Beach (12 miles north of Newport) yielded two specimens infected with the same rediae and cercariae. Lebour and Elmhirst (J. Mar. Biol. Ass. U. K., 20: 371–2) stated that the secondary host was either *Cardium edule* or *Mytilus edulis.* Dissection of 147 *Mytilus californianus* was made but no encysted metacercariae were observed. Stunkard and Cable fed cercariae that had encysted on the bottom of a finger bowl to young terns, guinea pigs, rats, and mice. The encysted cercariae developed only in the tern. Encysted cercariae were fed to one golden hamster (*Cricetus auratus*) and one mallard duck (*Anas platyrhynchos platyrhynchos*). The animals were dissected in 21 days; the encysted cercariae failed to develop in either animal.

Dawes stated that *Gymnophallus delicious* is found in the gall bladder of the common gull, herring gull, greater and lesser black-backed gull, and glaucous gull in Northern Europe. No other distribution records were found following a search through the literature. Finding this species in the Western gull at Newport represents new host and distribution records.

The work was conducted in the laboratory of Dr. Ivan Pratt.—Donald J. Reish, *Dept. of Zoology, Oregon State College, Corvallis, Oregon.*

USE OF A SURFACE ACTIVE AGENT TO PREVENT TRANSFER OF MALARIAL PARASITES BETWEEN BLOOD FILMS DURING MASS STAINING PROCEDURES

Brooke and Donaldson (1948, Pub. Hlth. Rep., 63: 991–1004) have reported that when thick blood films are stained together by mass procedures, blood elements may transfer from one film to another. If these transferring elements happen to contain malarial parasites and if they adhere to otherwise negative blood films, falsely positive diagnoses of malaria may result.

In an attempt to prevent or at least to reduce materially this transfer of blood elements during mass staining, various modifications of the staining procedure have been tested. Most of these modifications, which will be reported in detail elsewhere, did not eliminate the transfer. However, observations made during the experimental studies indicated that many of the blood elements which separated from the films float on the surface of the staining solution. It seemed logical to assume that if the surface tension of the solution could be reduced by the addition of a surface active agent the detached blood elements might sink to the bottom of the container and thus be less likely to adhere to other films.

As in the previously reported study, slides were bound together in groups of 25 or more according to the procedure described by Barber and Komp (1924, Proc. Internat. Cong. on Hlth. Prob. in Trop. Amer., United Fruit Co.). In each group of slides "normal" blood films (i.e., films obtained from individuals whose blood showed no malarial parasites and who had no history of malaria) were alternated with "positive" blood films (i.e., films obtained from paretics receiving malarial therapy or from a pigeon). The films were stained in glass containers of suitable size to accommodate the packages. In each experiment the test slides were stained for 45 minutes with a 1 to 50 dilution of Giemsa stain containing 0.5 per cent of a surface active agent, Triton X-30, which is a 33 per cent aqueous solution of an alkylated aryl poly-ether alcohol manufactured by Rohm and Haas Company, Philadelphia, Penn. (The trade name is carried as a means of identifying the product under discussion and does not represent endorsement of the product by

the Public Health Service.) The control slides were stained with diluted Giemsa containing no surface active agent. Nine experiments were performed, in seven of which the positive films were prepared from malarious blood and in two, from avian blood. None of the total of 225 normal films which were stained along with malarial blood films in Giemsa containing 0.5 per cent of the surface active agent showed any transferred parasites. On the other hand, 83 or 47.4 per cent of the 175 normal films stained along with malarial films in ordinary Giemsa stain showed transferred parasites. Normal films stained along with avian blood films in a Giemsa-Triton mixture showed a much lower transfer rate (6 per cent) than those stained in ordinary Giemsa (80 per cent).

At the concentration used in these studies the surface active agent appeared to have no unfavorable effects on the staining properties of the Giemsa solution. Moreover, the films which were stained with the Giemsa-Triton mixture were cleaner in appearance, thus facilitating microscopic examination. Additional studies are being made to determine the minimum amount of the surface active agent tested, as well as other surface active agents, which can be used and the effects of these agents upon the staining properties of the Giemsa solution.—M. M. Brooke and A. W. Donaldson, *Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Georgia.*

OCCURRENCE OF *DIPLOTRIAENA THOMASI* SEIBERT IN THE SLATE-COLORED JUNCO

Unknown *filariids* were obtained by Hudson S. Winn from specimens of *Junco hyemalis* which had died in captivity at Northwestern University, Evanston, Illinois. These nematodes were identified as *Diplotriaena thomasi* Seibert, 1944 (Trans. Amer. Micr. Soc. 63: 244–253). These worms were found in the body cavity in 3 out of the 36 birds autopsied. Each host had 44–46 worms. *D. thomasi* was previously known only from the white-throated sparrow, *Zonotrichia albicollis*. The measurements for *Diplotriaena* from the Junco are within the ranges of *D. thomasi* given by Seibert (1944).—Robert E. Ogren, *Department of Zoology and Physiology, University of Illinois, Urbana, Illinois.*

NOTE ON THE OCCURRENCE OF THE FLEA *NEARCTOPSYLLA HYRTACI* (ROTHSCHILD) IN THE UNITED STATES

Nearctopsylla hyrtaci was originally described in 1904 from specimens collected from a western mink, *"Putorius energumenos"* (*Mustela vison energumenos*), Cariboo district, British Columbia, and from a shrew, *Sorex obscurus*, also from British Columbia but without mention of locality. In view of the fact that to the present this rare species has been reported only from British Columbia, it is of interest to record its presence in the United States as evidenced by a male and female collected in western Montana by Mr. C. H. Conaway of the University of Montana, Missoula, who submitted them to the writer for identification. Both specimens were from *Sorex palustris* in Missoula County. According to information furnished by Mr. Conaway, the shrew on which the male was found was captured "at the edge of a ditch 100 feet from the Franklin Guard Station, T14N, R18W, Section 14, elevation 4200 feet, March 7, 1948." The female was found on a shrew captured "along Rattlesnake River, Greenough Park, Missoula, elevation 3200 feet, February 24, 1948."

The writer is grateful to Mr. Conaway for making the specimens available to him and for permission to publish the records.—Glen M. Kohls, *United States Public Health Service, Rocky Mountain Laboratory (Hamilton, Montana).*

OCCURRENCE OF THE TROPICAL RAT FLEA (*XENOPSYLLA CHEOPIS*) IN WYOMING

Records of *Xenopsylla cheopis* (Roths.) from the interior of the United States, as compiled by Becker (1947, Iowa Acad. Sci., 54: 297–300), show that this flea occurs in widely separated areas and is well established in the Middle West. Although Prince (1943, Pub. Hlth. Repts., 58: 700–708) reports it from Arizona, New Mexico, Colorado and Utah there are no records from Wyoming. An examination of domestic rats (*Rattus norvegicus* Erxleben) taken at Cheyenne, Wyoming on April 9, 1949, yielded two males and two females of *X. cheopis*. These findings suggest that this species of flea may be more common in the Rocky Mountain area than published records would indicate.—John S. Wiseman, *The University of Wyoming, Laramie, Wyoming.*

THE CHROMOSOMES OF *MACRACANTHORHYNCHUS HIRUDINACEUS* (PALLAS)

Specimens obtained from the intestine of freshly slaughtered hogs were fixed immediately after dissection in 0.85% saline. Fixatives used were Carnoy (6:3:1; alcohol: acetic: chloroform), Flemming (both "medium" and "strong"), Nawaschin (Randolph's modification and San Felice's modification), or Allen's B 15. Smears and sections were made of both male and female material, and stained with Heidenhain's iron haematoxylin, Meyer's (1945 stain technol. 20: 121–5) aceto-orcein, or Newton's (1929 J. Genetics 21: 1–16) crystal violet.

The chromosomes range in length from 8 microns to 16 microns in a nucleus of average size. The dividing cells of the female, found primarily in the ovarian masses, have one pair of large chromosomes with submedian centromeres, one pair of smaller chromosomes with median centromeres, and one pair of small chromosomes with subterminal centromeres. Thus there are two structurally identical sets of three, that being the haploid number. The dividing cells of the male, found in the testes of young specimens, show only one member of the pair of large chromosomes found in the female, the other member being replaced by a chromosome of greater relative size, bearing a median centromere. The other two pairs of chromosomes of the male are similar to those of the female.

Meyer (1933 Bronn's Klassen u. Ordn. des Tierr. 4: 464–7) indicated a varying chromosome number, which he explained as the result of frequent fusion of chromosomes, apparently in a random manner. No truly fused chromosomes were seen during the present study. It is reasonable to suppose that Meyer did not consider chromosome number to be as taxonomically important as most cytotaxonomists now believe it to be, and did not examine or prepare his material with this problem in mind.

The presence of heteromorphic chromosomes in the male suggests that these are sex-chromosomes. In that case the chromosome found only in the male might be designated "Y", and the pair of chromosomes with submedian centromeres, found in the female, "XX". Segregation of the tentative "Y" from its partner during meiosis is being investigated.—Arthur W. Jones and Helen L. Ward, *Department of Zoology and Entomology, University of Tennessee, Knoxville, Tennessee.*

GONGYLONEMA PULCHRUM IN THE BLACK BEAR, *EUARCTOS AMERICANUS*, AND THE PROBABLE SYNONYMY OF *G. PULCHRUM* MOLIN, 1857, WITH *G. URSI* (RUDOLPHI, 1819).

Through the kindness of J. Kenneth Doutt of the Carnegie Museum, the writer was sent the tongue of a black bear, *Euarctos americanus americanus*, collected by the Pennsylvania Mammal Survey (which is a Pittman-Robertson project, and is a cooperative feature between the Pennsylvania Game Commission, the U. S. Fish and Wildlife Service and the Carnegie Museum) in White Deer Valley, Lycoming County, Pa. The bear was in dying condition, extremely emaciated, with loose matted hair and easily torn skin.

The tongue was infected by numerous specimens of *Gongylonema*. Examination and measurements showed these worms to be similar in all respects to *G. pulchrum*, a species originally described by Molin (1857) from the European wild boar. Baylis (1925) showed that morphologically neither *G. scutatum* of ruminants or *G. ransomi* of American pigs were distinguishable from this species, and the identity of these species was subsequently confirmed by cross-infection experiments by Baylis, Sheather and Andrews (J. Trop. Med. Hyg. 29: 194–197, 346–349; 1926) and by Lucker (J. Parasit. 19: 134–141; 1932). *G. pulchrum* has little host specificity, having been obtained, naturally or experimentally, in pigs, various Bovidae, camel, horse, donkey, man, rat, guinea pigs and rabbits. In addition, as Baylis pointed out, there is no reason to believe that *G. spirale* Molin, 1857, from deer, is a distinct species.

Baylis and his co-workers made no mention of Rudolphi's (1819) *"Spiroptera ursi"* from the European brown bear, *Ursus arctos*, or Molin's (1860) *Gongylonema contortum* from the same host, which Neumann (1894) and Stossich (1897) considered synonyms. Lucker (l. c.) apparently overlooked these records from bears, since in connection with his failure to infect dogs with *G. pulchrum* of ruminant origin he remarked that carnivores are not known to harbor *Gongylonema*. The descriptions of these forms from European bears are very inadequate, based on specimens in the Vienna Museum; no modern description exists, and there is nothing in the existing descriptions to indicate any specific distinction from *G. pulchrum*. In view of the apparent rarity of these parasites in bears; the fact that they frequently locate themselves in the tongue instead of the esophagus, which Baylis et al (l. c.) pointed out may be due to their occurrence in an unnatural host; the known lack of host specificity of *G. pulchrum;* and the occurrence of a form indistinguishable from *G. pulchrum* in an American bear, it seems probable that the name

pulchrum Molin, 1857, will fall as a synonym of *ursi* Rudolphi, 1819. Before this change is made, however, the specimens in the Vienna Museum should be re-examined if they are still available.

Since the specific identity of the specimens here reported from *Euarctos americanus americanus* with *G. pulchrum* may have important taxonomic bearings, the following measurements are given. They should be compared with measurements of *Gongylonema* from ruminants and pigs given by Baylis (J. Trop. Med. Hyg. 28: 71–76; 1925).

Length of ♀, 41–55 mm; ♂, 20–25 mm. Diameter of body of ♀, 240–260 μ; of ♂, 180–190 μ. Length of esophagus of ♀ 7 mm, of ♂, 4–4.2 mm, with the anterior portion 500 to 660 μ. Tail of ♀ 200–250 μ; of ♂, 230–315 μ. Vulva 1.6 to 2.1 mm. from posterior end; left spicule 9–9.6 mm. long; right spicule 105 to 140 μ long; gubernaculum 50–70 μ long. ♂ with 5 pairs preanal papillae, and 4 pairs of large postanal papillae. Eggs 54–60 μ by 30–33 μ.—Asa C. Chandler, *Rice Institute, Houston, Tex.*

TRICHOSTRONGYLUS CALCARATUS IN MUSKRAT

Among some parasites kindly sent by J. Kenneth Doutt of the Carnegie Museum, collected by the Pennsylvania Mammal Survey (which is a Pittman-Robertson project, and is a cooperative feature between the Pennsylvania Game Commission, the U. S. Fish and Wildlife Service and the Carnegie Museum) was a vial containing about 25 male and female specimens of *Trichostrongylus calcaratus* from a muskrat, *Ondatra zibethicus*, collected in Crawford County, Pa. *T. calcaratus* is a very common and widely distributed parasite of rabbits in the United States, and was found in *Sylvilagus* from this same area. Graham and Uhrich (J. Parasit., 29: 159; 1943) found this parasite in 7 of 100 fox squirrels in Kansas, with an average of 7 worms per infection, and a maximum of 11 in a single infection. Rausch and Tiner (Amer. Midland Nat., 39: 728; 1948) reported it from squirrels from Wisconsin but not from Ohio or Michigan, and found only 1 or 2 specimens per host.

T. calcaratus, as far as the writer is aware, has not previously been reported from muskrats, but Barker, (J. Parasit., 1: 184; 1915) briefly described *T. fiberius* from muskrats in Nebraska. *T. fiberius* appears not to have been reported again, although there have been numerous investigations on muskrat parasites. Furthermore, there is nothing in Barker's description or in his illustrations by which *T. fiberius* can be differentiated from *T. calcaratus*. The name *T. fiberius* Barker and Noyes, 1915, is therefore considered a synonym of *T. calcaratus* Ransom, 1911.—Asa C. Chandler, *Rice Institute, Houston, Tex.*

MORPHOLOGICAL OBSERVATIONS ON THE ONCHOSPHERE OF *MESOCESTOIDES*

The onchosphere of a species of *Mesocestoides*, tentatively identified as *M. variabilis* Mueller, has been studied. Two thin, transparent membranes surround each hexacanth onchosphere in the parauterine organ. The medial hooks of the onchosphere are longer than the lateral hooks, and have a longer shank and hook. Detailed study of the morphology of the onchosphere permits the recognition of numerous somatic cells, 10–12 "plastin cells", and two penetration glands of a granular nature. Musculature for the movement of the hooks and body was observed as complex networks of a contractile nature. Flame cells of the type described for *Diphyllobothrium latum* and *Triaenophorus nodulosus*, have not been demonstrated. A more detailed description is being completed for publication.—Robert E. Ogren, *Department of Zoology and Physiology, University of Illinois, Urbana, Illinois.*

AN ADDITIONAL REPORT OF A REDUVIID BUG OTHER THAN *TRIATOMA* ATTACKING MAN

A Texas case of severe reaction to the injection of salivary secretions by a reduviid other than the *Triatoma* has recently come to our attention. Six adult bugs were sent to this department for identification October 28, 1948, from Dallas County, Texas. Mr. H. J. Reinhard, Entomology Department, Texas Agricultural and Mechanical College, stated that they are *Melanolestes picipes*. Two of the bugs were recovered from inside the shirt of an eight year old boy after he had been bitten five times. Intense pain was experienced for several hours; swelling at the puncture sites persisted for several days; pronounced ulcers remained for ten days. The most pronounced systemic reaction was an elevated temperature for twenty-four hours.

A search of the premises from which the insects were obtained revealed a moderate infestation of *M. picipes* which were probably attracted by light and warmth.—Richard B. Eads, *State Department of Health, Austin, Texas.*

ADDITIONAL HOSTS AND GEOGRAPHICAL DISTRIBUTION RECORDS FOR THE COMMON FISH ACANTHOCEPHALAN, *LEPTORHYNCHOIDES THECATUS*

Lincicome and Van Cleave (1949, Amer. Midl. Nat. 41: 421–431) have presented a geographical list of hosts of *Leptorhynchoides thecatus* based on their personal observations and the published literature. They reported this parasite from 79 fish hosts, 2 amphibian hosts, 2 reptile hosts, and 1 arthropod host. During the 1945 and 1946 parasite-surveys of northwest Wisconsin fishes, additional fish hosts recorded were *Boleosoma nigrum eulepis* (scaly Johnny darter) with encysted and mature worms, *Boleosoma nigrum eulepis* × *B. n. nigrum* (hybrid scaly × central Johnny darter) with encysted and mature worms, *Cottus b. bairdii* (northern muddler) with mature worms, *Eucalia inconstans* (brook stickleback) with encysted worms, *Hypentelium nigricans* (hog sucker) with encysted worms, *Osmerus mordax* (American smelt) with mature worms, and *Pungitius pungitius* (ninespine stickleback) with mature worms. The latter two species of fishes are Lake Superior forms collected at the mouth of the Brule river. Extension of the geographical range into northwest Wisconsin of *L. thecatus* in a known host is made for *Percina caprodes semifasciata* (northern logperch) with encysted and mature worms present; previously, this host was reported from Ohio, Michigan, and Lake Erie. Extension of the geographical range and a different stage of development of the parasite than recorded in a known host was made for *Lepomis cyanellus* (green sunfish) which contained mature worms; only encysted forms were recorded previously from Ohio. A different stage of development than heretofore recorded from a known host was the finding of encysted worms in *Fundulus diaphanus menona* (western banded killifish); previously, only mature forms were recorded from Minnesota and Wisconsin.—Jacob H. Fischthal, *Department of Biology, Triple Cities College of Syracuse University, Endicott, New York.*

DEEP-FREEZE PRESERVATION OF STOOL SPECIMENS CONTAINING INTESTINAL PARASITES

In view of the increasing use of deep-freeze lockers to preserve perishable materials for long periods of time, it was considered desirable to determine whether stool specimens containing parasites could be so preserved for shipping or storage purposes.

Fifty-seven normally passed stools containing cysts of intestinal amebae and *Giardia lamblia*, and 18 stools containing helminth eggs (primarily hookworm) were examined within 24 hours of passage and then frozen in a deep-freeze locker. After from 10 days to 3½ months, the specimens were allowed to thaw spontaneously and were re-examined by the same methods used before freezing.

The gross appearance of the thawed stools was similar to that of the freshly passed specimens. Microscopically, however, there was a considerable reduction in the numbers of cysts or eggs which could be seen or recovered by flotation techniques. Cysts were frequently distorted and difficult to identify both on direct examination and on $ZnSO_4$ flotation. Eggs were recovered by brine flotation in much smaller numbers than was possible before freezing. In addition, some of the eggs that did float were distorted.

It was concluded that the deep-freezing of stools is an unsatisfactory method for preserving cysts and eggs for subsequent demonstration by direct examination or by $ZnSO_4$ and brine flotation techniques.—Morris Goldman, S. A. and Sadie A. Johnson, *Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Ga.*

ATTEMPTS TO ADAPT *NIPPOSTRONGYLUS MURIS* TO THE COTTON RAT

It has been demonstrated that there is a low percentage development of *N. muris* in the cotton rat (Lindquist, unpublished thesis). The question arose as to whether this percentage could be increased by allowing the worm to develop through successive generations in the cotton rat under experimental conditions. An adaptive change has been shown to occur in the starling strain of *Syngamus trachea* in chickens by Taylor (1928, Ann. Trop. Med. and Parasit. 22: 307–318) and in dog and cat strains of *Ancylostoma caninum* by Scott (1930, Amer. J. Hyg. 11: 149–158). In both of these cases, however, the particular species of nematode is found in natural infections in the 2 hosts. Sheldon (1937, Jour. Parasit. 23: 98) suggested that *Strongyloides ratti* might be adapted to mice since he was able to establish light infections in 4 out of 12 mice. Brackett and Bliznick (1949, J. Parasit. 35: 41–44) tried this adaptation with *S. ratti* but found that after 19 passages there was no increase in adaptability. In this case the species of nematode is found naturally infecting only one of the hosts.

The present experiment was designed to check the possibility of adaptation of *N. muris* to cotton rats. Six litter mate cotton rats, 4 weeks old, were selected. Two of these were

given 7,500 larvae apiece, another two 5,000 larvae apiece and the remaining 2 received 2,500 larvae each. All infections were by subcutaneous injections. Daily fecal examinations were made on all 6 rats and as soon as any were positive, charcoal cultures were made on the daily collection of feces. At the end of 19 days all cultures of infective larvae were isolated by the Baermann apparatus. The total yield of larvae for all the cotton rats was a little over 8,000. The second generation of the nematode was started by giving 2 young cotton rats, 4 weeks old, 4,160 larvae each by the subcutaneous route. At the start of egg production in these 2 cotton rats all feces were cultured. Twenty-one days after infection the infective larvae cultures were isolated and the total return of larvae was 700. These larvae were injected into one young cotton rat and the feces examined for eggs. Having found no eggs by 19 days, the animal was autopsied. One adult male of *N. muris* was recovered. The conclusion was reached that this worm would not easily adapt itself to cotton rats in 3 generations. It should be possible to carry the parasite through more generations if more animals were initially infected with the highest possible number of larvae.—William D. Lindquist, *Department of Parasitology, School of Hygiene and Public Health, The Johns Hopkins University.*

ON THE STRUCTURE OF THE PARABASAL BODY IN *TRITRICHOMONAS BATRACHORUM* (PERTY) AND *TRITRICHOMONAS AUGUSTA* (ALEXEIEFF) OF AMPHIBIANS AND REPTILES

All the descriptions of the common trichomonad of Amphibia, *Tritrichomonas batrachorum*, fail to mention that this species differs from *Tritrichomonas augusta* not only in the structure of its axostyle, but also in that of its parabasal body. This latter difference can be observed easily in Protargol impregnations of intestinal smears, as well as of the culture-grown flagellates.

In contrast to the well known rod-like or sausage-shaped parabasal body of *Tritrichomonas augusta*, which tends to be located dorsal to the nucleus, that of *Tritrichomonas batrachorum* is "V" shaped and may be found frequently more to the right of the nucleus. The arms of the "V" are equal in length and width. Each of them continues in a fine filament. These filaments vary in length. It may be mentioned at this time that a similar filament can be found also in *Tritrichomonas augusta*, in which it sometimes reaches a considerable length. Probably in both of these species, as in many other trichomonads, the filaments run along the whole length of the parabasal body and then continue as free parabasal filaments. When the representatives of each of the two species correspond closely in over-all size and are fixed and impregnated under identical conditions, the arms of the parabasal apparatus of *Tritrichomonas batrachorum* do not differ appreciably in their width and length from the single parabasal body of *Tritrichomonas augusta*.

While a great deal of confusion exists in the taxonomy of the trichomonads of reptiles, it is certain that *Tritrichomonas augusta* is quite common in several species of lizards, whereas *Tritrichomonas batrachorum* is to be found in snakes and lizards alike. The differences noted in the parabasal apparatus of these two species of trichomonads from amphibians have been observed also in those from reptiles.—Bronislaw Honigberg, *Department of Zoology, University of California, Berkeley, California.*

EURYHELMIS SQUAMALA (RUDOLPHI), 1819 REPORTED FROM A RACOON

A racoon, *Procyon lotor*, from Durham Co., N. C. was examined on 22 March 1949. The duodenum was found to be heavily infected (several hundred specimens) with mature *Euryhelmis squamala* (Rudolphi), 1819. Specimens have been deposited in the U. S. National Museum Helminthological Collection (No. 46460). *E. squamala* is a parasite of the European polecat (Baer, J. G. 1931, Rev. Suisse Zool. 38: 313–334). The metacercaria of *E. squamala* were found in *Rana pipiens* near Alexandria, Virginia (McIntosh, A. 1936, J. Parasitol. 22: 536). Ameel, D. J. (1938, J. Parasitol. 24: 219–224) reported *E. monorchis* a closely related species in mink from Wisconsin and Michigan. No other records of *Euryhelmis* from North America have been found.—M. V. Parker, *Department of Zoology, Duke University, Durham, North Carolina.*

TRICHINIASIS IN ARKANSAS

A total of 155 medical students and medical technologists residing in various parts of the State have been examined for infection with *T. spiralis* by the intradermal test. Also, diaphragms from 27 unselected autopsies of Arkansas residents who died in the University Hospital were examined by the usual digestion methods.

Of the intradermal tests, two were found to be positive, one of which remained positive for about 12 hours. The reactions were obtained in white males 24 and 25 years old respectively, both living in Little Rock. Neither of the subjects recalled having had unusual gastric dis-

turbances or rheumatic symptoms suggesting clinical trichiniasis. In an additional case, both the antigen and the saline control produced an erythematous area of about one inch in diameter which persisted for about 30 minutes. Although the subject had a long history of sensitivity to various substances, the reaction with saline could not be explained.

Of the diaphragms examined, one was found to contain *T. spiralis* larvae in sufficient numbers to be detected by the common digestion method. The larvae observed were dead. The diaphragm came from a 40 year old colored female.

To the author's knowledge the only available figures on the occurrence of *T. spiralis* in Arkansas are those included in the report by Wright and his co-workers (1943, Pub. Health Rep. 58: 1293). They examined only two diaphragms and found them to be negative.

The State Health Department conducted examinations on 200 pig diaphragms. These were obtained from four slaughter houses in Little Rock and included pigs purchased throughout the State. No trichina larvae were recovered (data heretofore unpublished).

In view of the fact that the writer moved to another State, this survey had to be interrupted while the samples examined were still too few to afford any definite conclusion. They are reported here in order to make information available which, added to eventual future studies, may give some indication as to the degree of incidence of trichiniasis in Arkansas.

The author is indebted to Miss Glenda S. Cooper for her valuable technical assistance and to the Staff Members of the Department of Pathology, University of Arkansas, School of Medicine, for providing the diaphragms from autopsies—Elvio H. Sadun, *formerly with the University of Arkansas, School of Medicine, now with the Department of Tropical Medicine and Public Health, Tulane University, New Orleans, La.*

MESOSTEPHANUS LONGISACCUS, A NEW CYATHOCOTYLID TREMATODE FROM A DOG

Eight specimens of a small cyathocotylid fluke were collected from the small intestine of a dog obtained at the Houston dog pound. They conform to the characters of the genus *Mesostephanus* Lutz, 1935, except that no ventral sucker could be discerned in any of the eight specimens. In this respect they resemble members of the genus *Linstowiella* Szidat, 1933, but differ markedly from that genus in having a very highly developed cirrus pouch.

In all probability the dog is an accidental host. Most of the species of *Mesostephanus* have been found in fish-eating birds—gannets, cormorants, pelicans, frigate birds and night herons—but one, *M. appendiculatus*, was obtained in Roumania from dogs and cats experimentally fed fish containing metacercariae, and has since been reported from naturally infected dogs, once in the Ukraine and once from Washington, D. C. It is not unlikely that a water bird is the normal host for this species also. The lack of host specificity in this group is emphasized further by the closely related *Prohemistomum vivax*, a common parasite of kites in Egypt, which has been developed experimentally in dogs and cats, and has been recorded once from man. Possibly *Prosostephanus industrius*, found in dogs in China, will also prove to be normally parasitic in a fish-eating bird.

Specific description. Body 0.75 to 1.05 mm. long and 320 to 520μ broad, tongue-shaped, with a small, rounded, posterior appendage of variable size. Portion of body anterior to circle of vitelline follicles shorter than portion of body containing the holdfast and reproductive organs (average ratio about 42: 58). Oral sucker 72 to 80μ in diameter and 50 to 63μ long. Pharynx 50 to 75μ in diameter and 63 to 80μ long. No acetabulum observed. Esophagus short. Holdfast organ 290 to 360μ long and 200 to 250μ broad. Testes about 145 to 160μ in diameter, in tandem arrangement in dorsal part of body. Cirrus pouch very large, 340 to 410μ long with a maximum diameter, in its proximal part, of 80 to 90μ; anterior end reaches to or beyond middle of body length. Ovary not clearly seen, apparently having undergone degeneration. Vitellaria form border around holdfast organ except posteriorly, outer limits of border measuring 270 to 400μ in length and 270 to 325μ across. Eggs, in mounted specimens, 97 to 105μ by 65 to 67μ.

Host: dog

Location: small intestine

Locality: Houston, Texas

Type: U. S. Nat. Mus. Helm. Coll. No. 46467.

In addition to the absence of a ventral sucker, this worm differs from other species of *Mesostephanus* in the large size of the holdfast organ; the relatively great length of the cirrus pouch, which in most specimens has its proximal end anterior to the middle of the body; and in having the region of the body anterior to the reproductive organs occupy less than half the body length.

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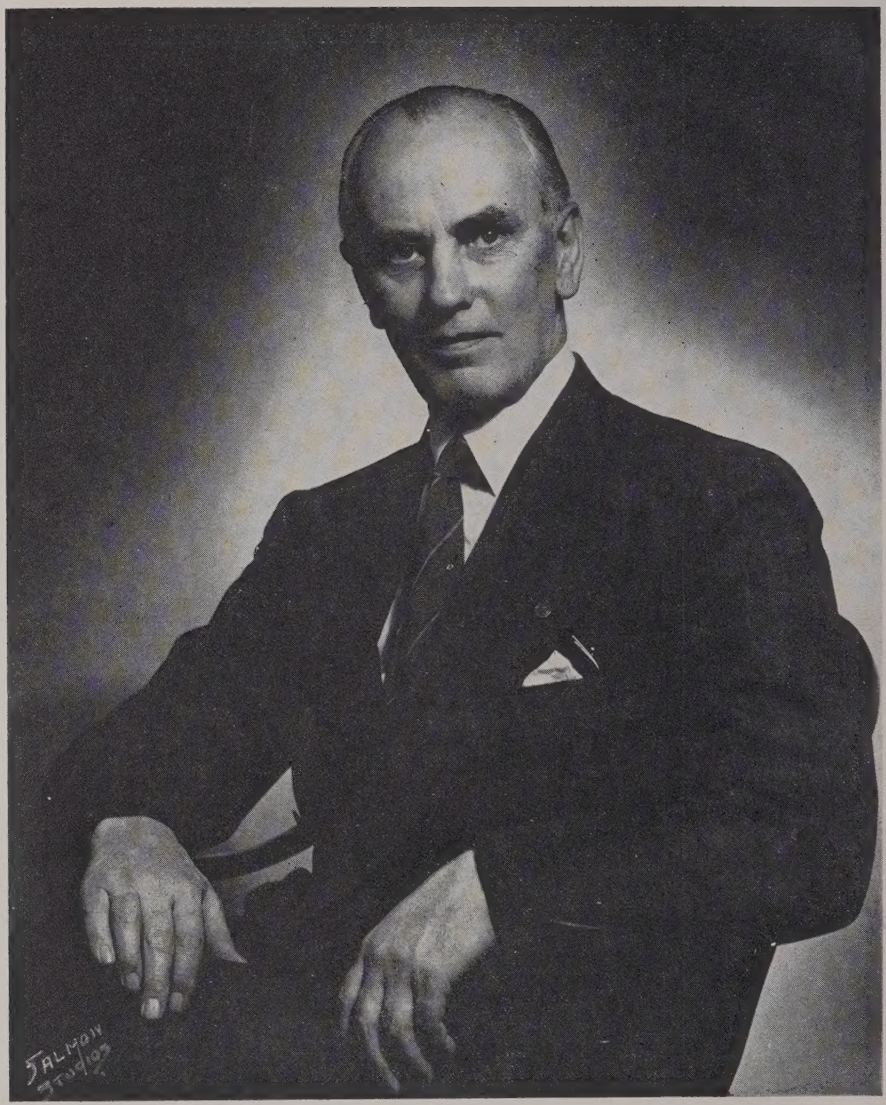
A NEW HOST FOR *CAPILLARIA CAUDINFLATA* (MOLIN, 1858).

Examination of some parasitic nematodes collected from a robin, *Turdus migratorius*, by Dr. Jack D. Tiner has revealed that these worms are specifically identical with *Capillaria caudinflata* (Molin, 1858). The bird was taken May 14, 1948, at Champaign, Illinois.

In recent years *C. caudinflata* has been reported from domestic galliform birds in various parts of the eastern half of North America. It has become increasingly apparent that this parasite has or is attaining a wide distribution on this continent. The report of Morehouse (1944, Ia. State Coll. J. Sci. 18: 217–253) of the experimental infection of the English sparrow, *Passer domesticus*, the recent report by the writer (1949, J. Parasit. *35*: 240–249) of a natural infection in a starling, *Sturnus vulgaris*, and the present report indicate a mode by which this worm may be introduced into previously uninfected flocks of domestic birds.—CLARK P. READ, *Department of Biology, The Rice Institute, Houston, Texas.*



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